Cresko Laboratory Procedures and Protocols

Cresko Laboratory

Thursday, January 18, 2024

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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 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.

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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.

5 Alizarin Staining

5.1 Introduction

- **Purpose**: Alizarin staining of fixed adult stickleback.
- **Procedure Type**: Molecular
- **Species**:
  - Threespine stickleback, *Gasterosteus aculeatus*
  - xxx
- **Authors**
  - xxx

> ⚠ Schedule for Cleaning
>
> PLACEHOLDER

5.2 Materials:

- Alizarin red S
- KOH
- H_2O_2
- NaOH
- MESAB
- PFA

5.3 Solutions:

- **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% H202/0.5%KOH**: Mix and keep at 4C; Before using, bring to room temperature to hold down introducing bubbles under the skin: 0.5ml 6%H202 & 0.5ml 1%KOH.
- MESAB: 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**:
  - 8 g Pelleted PFA (Ted Pella, Inc.; cat# 18501)
  - 90 ml dH2O
  - 25 drops 1N NaOH
  - Heat at very low heat and stir until solution clears.
  - Add 25 drops 1N HCl. pH should be 7.0-7.2.
  - Filter and store at 4C not more than 1 week.
  - Use as 4% PFA: dilute 1:1 with 2X PBS, do not store solution more than a few hours.
- **2X PBS**
  - 1.6% NaCl
  - 0.04% KCl
  - 0.04 M PO4 pH 7.0- 7.3

5.4 Procedure:

- **Day One**
  - 2h-8h at R/T depending on size 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)
- **Day Two**
  - xxx
  - xxx

– xxx

Part III

Microbiology

This ection of the book contains protocols that involve the use of microbes, mostly in the context of host-microbe interactions, but also standard protocols for cloning of nucleic acides using microbes.

6 Placeholder_Microbiology

6.1 xxx

xxxx

xxx

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.

7 Aquarium Cleaning

7.1 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

7.2 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).

7.3 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.

7.4 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.

7.5 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.

8 Artemia Decapsulation

8.1 Introduction

- **Purpose**: This procedure describes standard practices for decapsulating brine shrimp. Although brine shrimp can be hatched, collected and then fed to fish, the cysts are often hard to separate from the newly hatched brine shrimp and can be ingested by stickleback and pipefish. To reduce this phenomenon we can decapsulate brine shrimp in advance, and then leave them in a suspended state in the freezer for an extended period of time (~ xxx weeks) before they are hatched.
- **Procedure Type**: Husbandry
- **Species**:
  - Threespine stickleback, (*Gasterosteus aculeatus*),
  - Gulf pipefish (*Syngnathus scovelli*)
- **Authors**
  - Mark C. Currey

8.2 Materials:

- 15 oz can of dried Artemia cysts (approximately 430 g)
- 4.3 L ~6% laundry grade bleach
- Rock Salt (NaCl)
- 125 ml 40% Lye (NaOH) solution
- 30.0 g Sodium thiosulfate (Na_{2}S_{2}O_{3})
- 16 L Hatching Cone with aeration
- 125 ?m mesh bag (Aquatic Eco-Systems PMB3, 125 micron x 18")
- Several 3-5 L beakers
- (1-2) Squirt bottles - squeeze type

8.3 Solutions:

> ⚠ Be ready
>
> Solutions should be prepared in advance.

- Bleach, ~6% laundry grade
  - 25 ppt Salt Solution
  - Combine: 50 g Rock Salt (NaCl) To 2.0 L with tap water
  - Stir to dissolve completely.
- 40% Lye (NaOH) solution
  - Combine: 200 g Lye (NaOH) To 500 mL with tap water
  - Stir to dissolve completely.
  - Store in refrigerator (4°C)
- Buffered Salt Solution
  - Combine: 2L, 25 ppt Salt Solution
  - 125 mL 40% Lye Solution, pre-chilled to 4°C
- 1.0% Sodium Thiosulfate
  - Combine: 30 g sodium thiosulfate To 3.0 L with tap water
  - Stir to dissolve.
- Saturated Brine
  - Combine: ~25g Rock Salt to 4.0 L with tap water
  - Aerate to dissolve.

8.4 Procedure:

1. **Cyst hydration**: Hydrate one full can of dried cyst in 5 L of tap water in a hatching cone with aeration for 1 hour at room temp. Examine the cyst under a dissecting scope with top lighting before proceeding. Dry cysts are dimpled, resembling a deflated basketball, whereas fully hydrated cysts are completely spherical in shape. The cysts must be fully hydrated prior to the de-capsulation step. If cysts are not completely spherical after 1 hour, continue the hydration process (for a maximum of 2 hours), checking the progress of the cysts under a microscope every 15 min.
2. **Filter and rinse cysts**: Collect the hydrated cyst in a 125 um mesh bag and rinse with cool tap water.

3. **Transfer cysts back to the cone**: Add the Buffered Salt Solution to the cone and aerate (save back a filled squirt bottle of salt solution to help transfer cysts to cone). Transfer cysts into cone.

4. **De-capsulation**: Add the bleach (4.3 L) to the cone and continue aeration. Watch the cysts turn from brown to grey to orange, When the cysts are 90% orange, stop the reaction by quickly siphoning the cysts through a 125 um mesh bag and rinsing well with cool tap water.

5. **Neutralization residual chlorine**: To neutralize any residual chlorine transfer the mesh bag to a clean 4 L beaker and pour the 1.0% Sodium Thiosulfate (3L) into the bag. Soak the cysts in the sodium thiosulfate solution for ~1 min, then rinse the cysts with de-ionized tap water. Rinse until discharge turns clear.

6. **Dehydration for long-term storage**: Transfer the cysts back to the cone with 4 L of saturated brine and aerate until salt is dissolved. Transfer dehydrated cyst to (5 or 6) 1 L Nalgene bottles filled with 200 - 300 grams of salt. Add enough salt so that it does not dissolve when de-capsulated brine is added. Fill the bottles with de-capsulated brine. Store in refrigerator. The de-capsulated brine will store for at least 1 month. Hatch brine as you would capsulated brine (see Hatching and Feeding Brine SOP).

9 Syngnathid Euthanasia

9.1 Introduction

- **Purpose**: This procedure describes best practices for euthanizing embryonic and larval pipefish
- **Procedure Type**: Vertebrate Husbandry
- **Species**:
  - Bay pipefish, (*Syngnathus leptorhyncus*),
  - Gulf pipefish (*Syngnathus scovelli*)
- **Author**: xxx
- **Date Created**: xxx

> ⚠ NOTES
>
> xxxx

9.2 Materials

- Euthanasia strength Mesab (see above)
- Container to hold fish while being euthanized.
- Post mortem fixing equipment
- Morgue

9.3 Solutions

- MS-222 Stock solution of (4g/L, From Zebrafish Book 4th edition)??
  - Tricaine must be pharmaceutical-grade
  - We use tricaine purchased from Pentair, manufactured by Western Chemical and FDA approved.
  - Tricaine (3-amino benzoic acid ethyl lester also called ethyl m-aminoboenzoate) comes in a powdered form.

  - Purchase the smallest amount possible because tricaine expires quickly.

- Mesab Stock Solution (4g/L) (tris buffered):
  - 4 g tricaine powder
  - 979 ml DD water
  - ~21 ml 1 M Tris (pH 9).
  - Adjust pH to ~7.
  - Aliquot in 50ml tubes, label with MESAB Stock
  - Solution 4g/L, and store in a -20 freezer.
  - This makes 1 liter of solution.

- Euthanasia Solution (300 mg/L):
  - Make a solution of tris buffered Stock Solution as described above.
  - (Or obtain an aliquot from the freezer)
  - Combine 7.5ml of stock solution into 100 ml of fish water.

9.4 Procedure

- Procedure for embryo euthanasia:
  - Place embryos into a MS-222 euthanasia solution.
  - Leave embryos in solution until movement has stopped, ~ 10 minutes.
  - If fish is to be used for experiments, proceed with fixation or preparation for the experiment.
  - If the fish are to be disposed of, place embryo in 95% ETOH for 5 minutes and then into morgue for disposal.

> ⚠ NOTES
>
> This last step is necessary as a startle response is not obvious in unhatched fish

- Procedure for larval euthanasia:
  - Place larva into a MS-222 euthanasia solution.
  - Wait 10 minutes and perform a tap test to look for a startle response.
  - If there is a response wait 5 minutes and repeat startle response test.
  - Repeat this step every 5 minutes as needed.
  - If there is no response move to fixing for post mortem experiments or place in morgue.

Part V

Vertebrate Experiment

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

10 Alizarin Staining

Syngnathid fishes

10.1 Introduction

- **Purpose**: This procedure describes how to live alizarin stain pipefish
- **Procedure Type**: Amimal Experimental
- **Species**:
  - Bay pipefish, (*Syngnathus leptorhyncus*),
  - Gulf pipefish (*Syngnathus scovelli*)
- **Author**: xxx
- **Date Created**: xxx

> ⚠ NOTES
>
> xxxx

10.2 Solutions

- Alizarin stock solutions: 0.5g?Alizarin red in 100ml or in 50ml in sterile water?(SIGMA cat# A5533 Alizarin Red S, certified).?? ?
- Staining Solution: For 1 Liter:?
  - 990 ml embryo medium?(See embryo medium recipe).?
  - Add 10 ml 0.5% or 1% Alizarin Stock in sterile water?for final concentrations of 0.005% or 0.01%.
  - Adjust to pH 7.5 with NaOH ? For 50 ml (enough for 100mm diameter petri dish):?
- 49.5 ml Embryo medium?
- Add 500 ?l 0.5% or 1% Alizarin Stock in sterile water?
- Adjust to pH 7.5 with NaOH

10.3 Procedures

- Place fish into a container containing stain for 1-2 hours for larvae to overnight for juveniles or adult fish in the dark. We have found that fish do not experience adverse effects from being exposed to stain. Monitor fish every 30-60 minutes if possible. To de-stain, rinse thoroughly with embryo medium by placing fish into container of embryo medium without stain for 30 minutes; background continues to go down with time. Move on to DASPEI live staining if desired (see DASPEI live staining SOP) or anesthetize until the fish reaches a light plane of anesthesia (i.e. movement has slowed down enough that the fish can be safely handled) and observe/image.?
- Bone fluorescence will decrease over time, so plan on imaging the same day if possible.?
- Keep fish in the dark as much as is reasonably convenient.?
- After fish has been observed/imaged place in a container of fish water. Monitor fish every 5-10 minutes until the fish is revived. Once fish is revived place back on the fish system and monitor during daily health checks. If the fish is to be fixed for post mortem experiments place fish directly into euthanasia MS222 solution and follow the euthanasia SOP.

10.4 Associated Papers

11 Calcein staining

Syngnathid fishes

11.1 Introduction

- **Purpose**: This procedure describes how to live stain pipefish with calcein
- **Procedure Type**: Animal Experimental
- **Species**:
  - Bay pipefish, (*Syngnathus leptorhyncus*),
  - Gulf pipefish (*Syngnathus scovelli*)
- **Author**: xxx
- **Date Created**: xxx

> ⚠ NOTES
>
> xxxx

11.2 Materials:

- Petri dishes and/or 1 L tanks
- Calcein (Molecular Probes; cat. C481)

11.3 Solutions:

- MS-222 Anesthesia solution (see fish anesthesia and euthanasia SOP)?
- Artificial sea water
- 10% NaOH
- Stain Solution:
- 0.005 to 0.05% calcein in sea water.
- Adjust pH to 8.2 with NaOH
- Make fresh, keep in dark.

11.4 Procedures:

- Place fish into a container containing stain for 1-2 hours for larvae to overnight for juveniles or adult fish in the dark. We have found that fish do not experience adverse effects from being exposed to stain. Monitor fish every 30-60 minutes if possible. To de-stain, rinse thoroughly with embryo medium by placing fish into container of embryo medium without stain for 30 minutes; background continues to go down with time. Move on to DASPEI live staining if desired (see DASPEI live staining SOP) or anesthetize until the fish reaches a light plane of anesthesia (i.e. movement has slowed down enough that the fish can be safely handled) and observe/image.?
- Bone fluorescence will decrease over time, so plan on imaging the same day if possible.?
- Keep fish in the dark as much as is reasonably convenient.?
- After fish has been observed/imaged place in a container of fish water. Monitor fish every 5-10 minutes until the fish is revived. Once fish is revived place back on the fish system and monitor during daily health checks. If the fish is to be fixed for post mortem experiments place fish directly into euthanasia MS222 solution and follow the euthanasia SOP.

11.5 Associated Papers:

12 DASPEI staining

Syngnathid fishes

12.1 Introduction

- **Purpose**: This procedure describes how to live stain pipefish with DASPEI
- **Procedure Type**: xxx
- **Species**:
  - Bay pipefish, (*Syngnathus leptorhyncus*),
  - Gulf pipefish (*Syngnathus scovelli*)
- **Author**: Susan Bassham
- **Date Created**: 25 October 2021

> ⚠ xxxx
>
> xxxx

12.2 Materials:

- Petri dishes and/or 1 L tanks
- DASPEI (Sigma Aldrich; cat. D3418)

12.3 Solutions:

- MS-222 Anesthesia solution (see fish anesthesia and euthanasia SOP)?
- Artificial sea water

- Staining Solution: 0.005% DASPEI in sea water

12.4 Procedures:

- Stain larvae in Petri dishes and juveniles/adults in 1 L tanks containing stain solution for 5 to 75 min in the dark; stagger so that no fish stain longer than this before imaging. Rinse for 20 to 60 min in container of sea water without DASPEI to reduce background.?
- Keep fish in the dark as much as is reasonably convenient through procedures. Monitor fish every 15-30 minutes.?
- Anesthetize until the fish reaches a light plane of anesthesia (i.e. movement has slowed down enough that the fish can be safely handled) and observe/image fluorescence immediately.
- After fish has been observed/imaged place in a container of fish water. Monitor fish every 5-10 minutes until the fish is revived. Once fish is revived place back on the fish system and monitor during daily health checks. If the fish is to be fixed for post mortem experiments place fish directly into euthanasia MS222 solution and follow the euthanasia SOP.??
- Staining solution can be stored at 4C and reused.

12.5 Associated Papers

adapted from DOI: 10.1007/s10162-002-3022-x

13 Fin clipping

Syngnathid fishes

13.1 Introduction

- **Purpose**: This procedure describes how to take fin clips from live pipefish.
- **Procedure Type**: Husbandry
- **Species**:
  - Bay pipefish, (*Syngnathus leptorhyncus*),
  - Gulf pipefish (*Syngnathus scovelli*)
- **Author**: Mark C. Currey
- **Date Created**: 06 April 2010

> ⚠ xxxx
>
> xxxx

13.2 Solutions:

- MS-222 Anesthesia solution (see fish anesthesia and euthanasia SOP)
- Melafix (antifungal and antibacterial agent)

13.3 Materials:

- Beakers of system water
- Forceps and scissors
- Bucket of ice
- 1.5ml tubes

13.4 Procedure:

- To minimize fish?s exposure to MS-222, do all labeling of tubes and vials prior to fin clipping.
- Place fish in beaker containing MS-222 anesthesia dose in system water (168 mg/L).
- Once the breathing of the fish slows take fish out of water and remove caudal fin blade ? carefully avoiding the peduncle - with scissors and forceps. Note: To observe slowed breathing watch the operculum movement. This movement will slow and the fish will turn on its side.
- Place fin in labeled 1.5 ml tube on ice.
- Put fish into clean system water and move gently to force water over gills.
- Once fish is revived and swimming upright, put fish into new container with Melafix (diluted in system water according to manufacturer?s recommendations) for 15 minutes.
- Return fish to aquaculture system.

14 Syngnathid wild collections

14.1 Introduction

- **Purpose**: This procedure describes how to collect wild pipefish. Please note that new collectors should go with an experienced collector the first time to learn how to handle collecting devices and how to handle pipefish and by-catch.
- **Procedure Type**: Field
- **Species**:
  - Bay pipefish, (*Syngnathus leptorhyncus*),
  - Gulf pipefish (*Syngnathus scovelli*)
- **Author**: Mark Currey
- **Date Created**: September 10, 2012

14.2 Materials

- Minnow Traps
- Seine
- Dip nets
- Field Notebook
- 500 and 1000ml Nalgene bottles
- Bucket
- Net
- Mouse cage
- Gloves

> ⚠ OPTIONAL
>
> - Waders
> - Kayak

14.3 Solutions

- 95% ETOH
- Mesab (see fish Euthanasia SOP)

14.4 Procedure

- **Minnow trap collecting**
  - Place traps in areas with cover.
  - Tether trap to shore or other non-movable object.
  - Leave traps up to but not over 24 hours. Traps may need to be checked more frequently depending on the tidal influence of water body being investigated.
  - Pull traps looking for endangered species such as salmonids, Oregon chub, and/or Bull Trout. If an endangered species is noticed open trap under water and release all of the fish.
  - If no endangered species are noticed, remove trap from the water and empty contents in a bucket filled 1/3 with water from where the trapping is taking place.
  - Remove, count, and record all pipefish and non-pipefish species. This will be used for ODFW reporting at the end of the year.
  - Record (in field notebook); collecting location, GPS coordinates, water temp, water type, water condition, substrate condition, vegetation condition, pH (if possible), D.O. (if possible), and other environmental measurements.
  - Use dip net and/or siene to capture fish, return all non-targeted species immediately back into the water.

> ⚠ TRAP PLACEMENT
>
> Care should be taken when setting traps in tidally influenced waters as traps that are set above the low water line have the potential to leave fish without water resulting in fish fatality.

- **Trap Disinfection**:
  - Let minnow traps and other collecting equipment air dry for ~ 1 week between collecting locations.
  - Further steps may be needed depending on the invasive species encountered. Updates will come soon.

> ⚠ TRAP DISINFECTION
>
> When trapping between watersheds or between different water bodies within a watershed that ODFW has requested that we disinfect our traps and collecting equipment (see comments for current ODFW fish take permit) please do the following.

- **Euthanize and Storage of Collected Fish**:
  - Euthanize fish using MS222 (see Fish Euthanasia and Anesthesia SOP)
  - Collect fish by pouring fish through a net. MS222 can be collected by pouring solution into a secondary container.
  - Place fish into a Nalgene container and fill with 95% ETOH. Do not fill bottle over half full with fish as fluids coming out of fish can dilute ETOH to the point that DNA/Tissue degradation can take place.
  - Return to lab and assign stock #.
  - Alternatively, euthanized fish can be flash frozen by placing in liquid nitrogen and stored in dry ice if high quality DNA is required.

14.5 Associated Papers

- xxx
- xxx
- xxx

15 Stickleback wild collections

15.1 Introduction

- **Purpose**: This procedure describes how to collect wild threespine stickleback.
- **Procedure Type**: Field
- **Species**: Threespine stickleback, (*Gasterosteus aculeatus*)
- **Author**: Mark Currey
- **Date Created**: September 10, 2012

15.2 Materials

- Minnow Traps
- Field Notebook
- 500 and 1000ml Nalgene bottles
- Bucket
- Net
- Mouse cage
- Gloves
- Crossing materials (see stickleback crossing SOP)

> ⚠ OPTIONAL
>
> - Waders
> - Kayak

15.3 Solutions

- 95% ETOH
- Mesab (see fish Euthanasia SOP)

15.4 Procedure

- **Minnow trap collecting**

    1. Place traps in areas with cover.
    2. Tether trap to shore or other non-movable object.
    3. Leave traps up to but not over 24 hours. Traps may need to be checked more frequently depending on the tidal influence of water body being investigated.
    4. Pull traps looking for endangered species such as salmonids, Oregon chub, and/or Bull Trout. If an endangered species is noticed open trap under water and release all of the fish.
    5. If no endangered species are noticed, remove trap from the water and empty contents in a bucket filled 1/3 with water from where the trapping is taking place.
    6. Remove, count, and record all stickleback and non-stickleback species. This will be used for ODFW reporting at the end of the year.
    7. Record (in field notebook); collecting location, GPS coordinates, water temp, water type, water condition, substrate condition, vegetation condition, pH (if possible), D.O. (if possible), and other environmental measurements.

> ⚠ TRAP PLACEMENT
>
> Care should be taken when setting traps in tidally influenced waters as traps that are set above the low water line have the potential to leave fish without water resulting in fish fatality.

- **Trap Disinfection**:

    1. Let minnow traps and other collecting equipment air dry for ~ 1 week between collecting locations.
    2. Further steps may be needed depending on the invasive species encountered. Updates will come soon.

> ⚠ TRAP DISINFECTION
>
> When trapping between watersheds or between different water bodies within a watershed that ODFW has requested that we disinfect our traps and collecting equipment (see comments for current ODFW fish take permit) please do the following.

- **Euthanize and Storage of Collected Fish**:

1. Euthanize fish using MS222 (see Fish Euthanasia and Anesthesia SOP)
2. Collect fish by pouring fish through a net. MS222 can be collected by pouring solution into a secondary container.
3. Place fish into a Nalgene container and fill with 95% ETOH. Do not fill bottle over half full with fish as fluids coming out of fish can dilute ETOH to the point that DNA/Tissue degradation can take place.
4. Return to lab and assign stock #.
5. Alternatively, euthanized fish can be flash frozen by placing in liquid nitrogen and stored in dry ice if high quality DNA is required.

- **Wild crosses introduction into the lab**:
  - Crosses fish (see stickleback crossing SOP).
  - Bleach embryos (see embryo bleaching SOP).
  - Introduce into fish system (see stickleback crossing SOP).
  - If embryos are to be fixed and used for investigation fix embryos (see Embryo and larval euthanasia SOP).

15.5 Associated Papers

- xxx
- xxx
- xxx

Part VI

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*

16 Placeholder_Daphnia

16.1 xxx

xxxx

xxx

xxxxx

Part VII

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.

17 A field guide to base R

17.1 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)
```

17.2 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.

17.3 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.

Part VIII

References&Notes

This section of the book contains references and notes for all protocols

References

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

Notes

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	ACACTCTTTCCCTACACGACGCTCTTC[illegible]TGC*A	A1	SbfI-AAACGG-bot	/5Phos/[illegible]AGAAGAGCGT[illegible]
A2	AACGTT	SbfI-AACGTT-top	ACACTCTTTCCCTACACGACGCTCTTC[illegible]TGC*A	A2	SbfI-AACGTT-bot	/5Phos/[illegible]AGAAGAGCGT[illegible]
A3	AACTGA	SbfI-AACTGA-top	ACACTCTTTCCCTACACGACGCTCTTC[illegible]TGC*A	A3	SbfI-AACTGA-bot	/5Phos/[illegible]AGAAGAGCGT[illegible]
A4	AAGACG	SbfI-AAGACG-top	ACACTCTTTCCCTACACGACGCTCTTC[illegible]TGC*A	A4	SbfI-AAGACG-bot	/5Phos/[illegible]AGAAGAGCGT[illegible]
A5	AAGCTA	SbfI-AAGCTA-top	ACACTCTTTCCCTACACGACGCTCTTC[illegible]TGC*A	A5	SbfI-AAGCTA-bot	/5Phos/[illegible]AGAAGAGCGT[illegible]
A6	AATATC	SbfI-AATATC-top	ACACTCTTTCCCTACACGACGCTCTTC[illegible]TGC*A	A6	SbfI-AATATC-bot	/5Phos/[illegible]AGAAGAGCGT[illegible]
A7	AATGAG	SbfI-AATGAG-top	ACACTCTTTCCCTACACGACGCTCTTC[illegible]TGC*A	A7	SbfI-AATGAG-bot	/5Phos/[illegible]AGAAGAGCGT[illegible]
A8	ACAAGA	SbfI-ACAAGA-top	ACACTCTTTCCCTACACGACGCTCTTC[illegible]TGC*A	A8	SbfI-ACAAGA-bot	/5Phos/[illegible]AGAAGAGCGT[illegible]
A9	ACAGCG	SbfI-ACAGCG-top	ACACTCTTTCCCTACACGACGCTCTTC[illegible]TGC*A	A9	SbfI-ACAGCG-bot	/5Phos/[illegible]AGAAGAGCGT[illegible]
A10	ACATAC	SbfI-ACATAC-top	ACACTCTTTCCCTACACGACGCTCTTC[illegible]TGC*A	A10	SbfI-ACATAC-bot	/5Phos/[illegible]AGAAGAGCGT[illegible]

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
A11	ACCATG	SbfI-ACCATG-top	ACACTCTTTCCCTACACGACGCTCTTC...	A11	SbfI-ACCATG-bot	/5Phos/CATGGTAGATCGGAAGAGCG...
A12	ACCCCC	SbfI-ACCCCC-top	ACACTCTTTCCCTACACGACGCTCTTC...	A12	SbfI-ACCCCC-bot	/5Phos/GGGGGTAGATCGGAAGAGCG...
B1	ACTCTT	SbfI-ACTCTT-top	ACACTCTTTCCCTACACGACGCTCTTC...	B1	SbfI-ACTCTT-bot	/5Phos/AAGAGTAGATCGGAAGAGCG...
B2	ACTGGC	SbfI-ACTGGC-top	ACACTCTTTCCCTACACGACGCTCTTC...	B2	SbfI-ACTGGC-bot	/5Phos/GCCAGTAGATCGGAAGAGCG...
B3	AGCCAT	SbfI-AGCCAT-top	ACACTCTTTCCCTACACGACGCTCTTC...	B3	SbfI-AGCCAT-bot	/5Phos/ATGGCTAGATCGGAAGAGCG...
B4	AGCGCA	SbfI-AGCGCA-top	ACACTCTTTCCCTACACGACGCTCTTC...	B4	SbfI-AGCGCA-bot	/5Phos/TGCGCTAGATCGGAAGAGCG...
B5	AGGGTC	SbfI-AGGGTC-top	ACACTCTTTCCCTACACGACGCTCTTC...	B5	SbfI-AGGGTC-bot	/5Phos/GACCCTAGATCGGAAGAGCG...
B6	AGGTGT	SbfI-AGGTGT-top	ACACTCTTTCCCTACACGACGCTCTTC...	B6	SbfI-AGGTGT-bot	/5Phos/ACACCTAGATCGGAAGAGCG...
B7	AGTAGG	SbfI-AGTAGG-top	ACACTCTTTCCCTACACGACGCTCTTC...	B7	SbfI-AGTAGG-bot	/5Phos/CCTACTAGATCGGAAGAGCG...
B8	AGTTAA	SbfI-AGTTAA-top	ACACTCTTTCCCTACACGACGCTCTTC...	B8	SbfI-AGTTAA-bot	/5Phos/TTAACTAGATCGGAAGAGCG...
B9	ATAGTA	SbfI-ATAGTA-top	ACACTCTTTCCCTACACGACGCTCTTC...	B9	SbfI-ATAGTA-bot	/5Phos/TACTATAGATCGGAAGAGCG...
B10	ATCAAA	SbfI-ATCAAA-top	ACACTCTTTCCCTACACGACGCTCTTC...	B10	SbfI-ATCAAA-bot	/5Phos/TTTGATAGATCGGAAGAGCG...
B11	ATGCAC	SbfI-ATGCAC-top	ACACTCTTTCCCTACACGACGCTCTTC...	B11	SbfI-ATGCAC-bot	/5Phos/GTGCATAGATCGGAAGAGCG...

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
B12	ATGTTG	SbfI-ATGTTG-top	ACACTCTTTCCCTACACGACGCTCTTC	B12	SbfI-ATGTTG-bot	/5Phos/CAACATAGATCGGAAGAGCG
C1	ATTCCG	SbfI-ATTCCG-top	ACACTCTTTCCCTACACGACGCTCTTC	C1	SbfI-ATTCCG-bot	/5Phos/CGGAATAGATCGGAAGAGCG
C2	CAAAAA	SbfI-CAAAAA-top	ACACTCTTTCCCTACACGACGCTCTTC	C2	SbfI-CAAAAA-bot	/5Phos/TTTTTGAGATCGGAAGAGCG
C3	CAATCG	SbfI-CAATCG-top	ACACTCTTTCCCTACACGACGCTCTTC	C3	SbfI-CAATCG-bot	/5Phos/CGATTGAGATCGGAAGAGCG
C4	CACCTC	SbfI-CACCTC-top	ACACTCTTTCCCTACACGACGCTCTTC	C4	SbfI-CACCTC-bot	/5Phos/GAGGTGAGATCGGAAGAGCG
C5	CAGGCA	SbfI-CAGGCA-top	ACACTCTTTCCCTACACGACGCTCTTC	C5	SbfI-CAGGCA-bot	/5Phos/TGCCTGAGATCGGAAGAGCG
C6	CATACT	SbfI-CATACT-top	ACACTCTTTCCCTACACGACGCTCTTC	C6	SbfI-CATACT-bot	/5Phos/AGTATGAGATCGGAAGAGCG
C7	CCATTT	SbfI-CCATTT-top	ACACTCTTTCCCTACACGACGCTCTTC	C7	SbfI-CCATTT-bot	/5Phos/AAATGGAGATCGGAAGAGCG
C8	CCCGGT	SbfI-CCCGGT-top	ACACTCTTTCCCTACACGACGCTCTTC	C8	SbfI-CCCGGT-bot	/5Phos/ACCGGGAGATCGGAAGAGCG
C9	CCCTAA	SbfI-CCCTAA-top	ACACTCTTTCCCTACACGACGCTCTTC	C9	SbfI-CCCTAA-bot	/5Phos/TTAGGGAGATCGGAAGAGCG
C10	CCGAGG	SbfI-CCGAGG-top	ACACTCTTTCCCTACACGACGCTCTTC	C10	SbfI-CCGAGG-bot	/5Phos/CCTCGGAGATCGGAAGAGCG
C11	CCGCAT	SbfI-CCGCAT-top	ACACTCTTTCCCTACACGACGCTCTTC	C11	SbfI-CCGCAT-bot	/5Phos/ATGCGGAGATCGGAAGAGCG
C12	CCTAAC	SbfI-CCTAAC-top	ACACTCTTTCCCTACACGACGCTCTTC	C12	SbfI-CCTAAC-bot	/5Phos/GTTAGGAGATCGGAAGAGCG

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
D1	CGAGGC	SbfI-CGAGGC-top	ACACTCTTTCCCTACA[illegible]	D1	SbfI-CGAGGC-bot	/5Phos/[illegible]AGAAGAGCG
D2	CGCAGA	SbfI-CGCAGA-top	ACACTCTTTCCCTACA[illegible]	D2	SbfI-CGCAGA-bot	/5Phos/[illegible]AGAAGAGCG
D3	CGCGTG	SbfI-CGCGTG-top	ACACTCTTTCCCTACA[illegible]	D3	SbfI-CGCGTG-bot	/5Phos/[illegible]AGAAGAGCG
D4	CGGTCC	SbfI-CGGTCC-top	ACACTCTTTCCCTACA[illegible]	D4	SbfI-CGGTCC-bot	/5Phos/[illegible]AGAAGAGCG
D5	CGTCTA	SbfI-CGTCTA-top	ACACTCTTTCCCTACA[illegible]	D5	SbfI-CGTCTA-bot	/5Phos/[illegible]AGAAGAGCG
D6	CGTGAT	SbfI-CGTGAT-top	ACACTCTTTCCCTACA[illegible]	D6	SbfI-CGTGAT-bot	/5Phos/[illegible]AGAAGAGCG
D7	CTACAG	SbfI-CTACAG-top	ACACTCTTTCCCTACA[illegible]	D7	SbfI-CTACAG-bot	/5Phos/[illegible]AGAAGAGCG
D8	CTCGCC	SbfI-CTCGCC-top	ACACTCTTTCCCTACA[illegible]	D8	SbfI-CTCGCC-bot	/5Phos/[illegible]AGAAGAGCG
D9	CTGCGA	SbfI-CTGCGA-top	ACACTCTTTCCCTACA[illegible]	D9	SbfI-CTGCGA-bot	/5Phos/[illegible]AGAAGAGCG
D10	CTGGTT	SbfI-CTGGTT-top	ACACTCTTTCCCTACA[illegible]	D10	SbfI-CTGGTT-bot	/5Phos/[illegible]AGAAGAGCG
D11	CTTATG	SbfI-CTTATG-top	ACACTCTTTCCCTACA[illegible]	D11	SbfI-CTTATG-bot	/5Phos/[illegible]AGAAGAGCG
D12	CTTTGC	SbfI-CTTTGC-top	ACACTCTTTCCCTACA[illegible]	D12	SbfI-CTTTGC-bot	/5Phos/[illegible]AGAAGAGCG
E1	GAAATG	SbfI-GAAATG-top	ACACTCTTTCCCTACA[illegible]	E1	SbfI-GAAATG-bot	/5Phos/[illegible]AGAAGAGCG

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
E2	GAACCA	SMI-GAACCA-top	ACACTCTTTCCCTACACG	E2	SMI-GAACCA-bot	/5Phos/CTGGATCCAGTC*G
E3	GACGAC	SMI-GACGAC-top	ACACTCTTTCCCTACACG	E3	SMI-GACGAC-bot	/5Phos/CTGAGTCCAGTC*G
E4	GACTCT	SMI-GACTCT-top	ACACTCTTTCCCTACACG	E4	SMI-GACTCT-bot	/5Phos/CAGAGTCCAGTC*G
E5	GAGAGA	SMI-GAGAGA-top	ACACTCTTTCCCTACACG	E5	SMI-GAGAGA-bot	/5Phos/CTCTACCAGTC*G
E6	GATCGT	SMI-GATCGT-top	ACACTCTTTCCCTACACG	E6	SMI-GATCGT-bot	/5Phos/ACGATCCAGTC*G
E7	GCAGAT	SMI-GCAGAT-top	ACACTCTTTCCCTACACG	E7	SMI-GCAGAT-bot	/5Phos/ATCTGCCAGTC*G
E8	GCATGG	SMI-GCATGG-top	ACACTCTTTCCCTACACG	E8	SMI-GCATGG-bot	/5Phos/CCATGCCAGTC*G
E9	GCCGTA	SMI-GCCGTA-top	ACACTCTTTCCCTACACG	E9	SMI-GCCGTA-bot	/5Phos/TACGGCCAGTC*G
E10	GCGACC	SMI-GCGACC-top	ACACTCTTTCCCTACACG	E10	SMI-GCGACC-bot	/5Phos/GGTCGCCAGTC*G
E11	GCGCTG	SMI-GCGCTG-top	ACACTCTTTCCCTACACG	E11	SMI-GCGCTG-bot	/5Phos/CAGCGCCAGTC*G
E12	GCTCAA	SMI-GCTCAA-top	ACACTCTTTCCCTACACG	E12	SMI-GCTCAA-bot	/5Phos/TTGAGCCAGTC*G
F1	GGACTT	SMI-GGACTT-top	ACACTCTTTCCCTACACG	F1	SMI-GGACTT-bot	/5Phos/AAGTCCCAGTC*G
F2	GGCAAG	SMI-GGCAAG-top	ACACTCTTTCCCTACACG	F2	SMI-GGCAAG-bot	/5Phos/CTTGCCCAGTC*G

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
F3	GGGCGC	SbfI-GGGCGC-top	ACACTCTTTCCCTACACG	F3	SbfI-GGGCGC-bot	/5Phos/CTGGCCCACGTGTC*G
F4	GGGGCG	SbfI-GGGGCG-top	ACACTCTTTCCCTACACG	F4	SbfI-GGGGCG-bot	/5Phos/CTGGCCCCGCGTGTC*G
F5	GGTACA	SbfI-GGTACA-top	ACACTCTTTCCCTACACG	F5	SbfI-GGTACA-bot	/5Phos/CTGGTACTGCGTGTC*G
F6	GGTTTG	SbfI-GGTTTG-top	ACACTCTTTCCCTACACG	F6	SbfI-GGTTTG-bot	/5Phos/CTGAACTCAGGTGTC*G
F7	GTAAGT	SbfI-GTAAGT-top	ACACTCTTTCCCTACACG	F7	SbfI-GTAAGT-bot	/5Phos/CTGTTACTTGGTGTC*G
F8	GTATCC	SbfI-GTATCC-top	ACACTCTTTCCCTACACG	F8	SbfI-GTATCC-bot	/5Phos/CTGATACGGGGTGTC*G
F9	GTCATC	SbfI-GTCATC-top	ACACTCTTTCCCTACACG	F9	SbfI-GTCATC-bot	/5Phos/CTGTGACTAGGTGTC*G
F10	GTGCCT	SbfI-GTGCCT-top	ACACTCTTTCCCTACACG	F10	SbfI-GTGCCT-bot	/5Phos/CTGGCACCAGGTGTC*G
F11	GTGTAA	SbfI-GTGTAA-top	ACACTCTTTCCCTACACG	F11	SbfI-GTGTAA-bot	/5Phos/CTGTCATTAGGTGTC*G
F12	GTTGGA	SbfI-GTTGGA-top	ACACTCTTTCCCTACACG	F12	SbfI-GTTGGA-bot	/5Phos/CTGCTAACCAGTGTC*G
G1	TAAGCT	SbfI-TAAGCT-top	ACACTCTTTCCCTACACG	G1	SbfI-TAAGCT-bot	/5Phos/CTGTAGCAGCGTGTC*G
G2	TAATTC	SbfI-TAATTC-top	ACACTCTTTCCCTACACG	G2	SbfI-TAATTC-bot	/5Phos/CTGTAATTAGGTGTC*G
G3	TACACA	SbfI-TACACA-top	ACACTCTTTCCCTACACG	G3	SbfI-TACACA-bot	/5Phos/CTGTGTATGGGTGTC*G

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

well	Barcode	Name (top)	Final top sequence	well	Name (bottom)	Final bottom sequence
H5	TGCTTA	SbfI-TGCTTA-top	ACACTCTTTCCCTACACG	H5	SbfI-TGCTTA-bot	/5Phos/CTAAGCTAAGTC*GAAGAGCGT
H6	TGGGGA	SbfI-TGGGGA-top	ACACTCTTTCCCTACACG	H6	SbfI-TGGGGA-bot	/5Phos/CTCCCCAAGTC*GAAGAGCGT
H7	TTATGA	SbfI-TTATGA-top	ACACTCTTTCCCTACACG	H7	SbfI-TTATGA-bot	/5Phos/CTCATAAGGTC*GAAGAGCGT
H8	TTCCGT	SbfI-TTCCGT-top	ACACTCTTTCCCTACACG	H8	SbfI-TTCCGT-bot	/5Phos/CACGGAAGTC*GAAGAGCG
H9	TTCTAG	SbfI-TTCTAG-top	ACACTCTTTCCCTACACG	H9	SbfI-TTCTAG-bot	/5Phos/CTAGAAAGTC*GAAGAGCGT
H10	TTGAGC	SbfI-TTGAGC-top	ACACTCTTTCCCTACACG	H10	SbfI-TTGAGC-bot	/5Phos/CGCTCAAGTC*GAAGAGCG
H11	TTTAAT	SbfI-TTTAAT-top	ACACTCTTTCCCTACACG	H11	SbfI-TTTAAT-bot	/5Phos/CATTAAAGTC*GAAGAGCGT
H12	TTTGTC	SbfI-TTTGTC-top	ACACTCTTTCCCTACACG	H12	SbfI-TTTGTC-bot	/5Phos/CGACAAAGTC*GAAGAGCG

B Gel electrophoresis tips

B.1 Introduction

- **Purpose**: To help members of the laboratory run the perfect gels - every time.
- **Procedure Type**: Molecular
- **Species**: N/A
- **Authors**
  - Susan Bassham

The effect of electrophoresis is to separate DNA fragments by size in an agarose matrix and buffer using an electrical current. DNA is negatively charged and will migrate toward the positive pole. Many parameters can affect how the DNA moves through the gel: buffer composition, voltage, length of the gel, percentage of agarose in the gel (in other words, the density of the gel matrix), presence of salt in the DNA, protein bound to DNA, and other factors. Some of these conditions also affect which size ranges of DNA will effectively be "resolved" (i.e., separated enough from one another so you can see them as distinct fragment sizes). "Safeview" is a dye we use in the gel that fluoresces under UV or blue light when bound to DNA, allowing us to see and photograph it when viewed through a special orange filter to cut out the background light. The research goals of using electrophoresis might include:

- measuring the size of fragments in a DNA sample such as the products of PCR or assessing the intactness of the purified DNA from a tissue extraction
- separating fragments from one another so that a particular amplicon or size range of DNA can be purified out of the gel for other downstream applications (such as for cloning a PCR fragment for making probes or for Sanger sequencing, or for size selecting a smear of fragments for making a RAD library) while excluding the other fragment sizes
- checking for the presence or absence of a particular product of PCR as in a screen for orientation of a cloned fragment in a plasmid present in different bacterial colonies, a screen for an insertion in a transgenic animal, a screen to determine the genetic sex or the mitotype of a fish.

B.2 Common Mistakes

> ⚠ Warning
>
> These common mistakes will give you a big headache when trying to run gels

- **Boiling over the agarose when making a gel**.
  - *Result*: failure to monitor the agarose when you are trying to dissolve it in the microwave can easily cause it to boil over because powdered substances create a lot of "nodes of nucleation" for bubbles to form. This is especially easy to accidentally do with higher percentages of agarose. It wastes expensive agarose and creates a mess in the microwave. What remains in the bottle will be of an indeterminate percentage/gel stiffness.
- **Not making sure the tape is adhering well to the mold before casting a gel**.
  - *Result*: liquid agarose will leak out causing a mess and reagent waste. Make sure the mold is dry beforehand, and use the back edge of a comb, e.g., to run across the tape and make sure it has firm contact.
- **Positioning the comb teeth too close to one side or to the bottom of the mold when setting up for pouring a gel**.
  - *Result*: the bottoms (or that side) of wells may be torn when the comb is pulled out, causing the loaded samples to leak out into the buffer during gel loading. Note: even when the comb teeth are not too close to the bottom, low percentage gels are soft and will benefit from having the comb pulled while submerged in the tank - otherwise the suction created by surface tension between the comb plastic and the agarose as you pull up on the comb can tear the bottoms out of the wells, causing loss of samples during loading.
- **Not using the right % of agarose for your DNA size range of interest**.
  - *Result*: bands you care about won't resolve optimally (i.e., you might not be able to accurately measure the sizes of your bands and might not be able to tell if something is one band or multiple bands). Pour a higher percentage gel to resolve large fragments (e.g. less than 1%) or a lower percentage gel (i.e. between 1 and 4%) to resolve small to very small ones, depending on your needs/expectations for what size bands you will see, how many of them, or how important it is to accurately estimate their sizes.
- **Not homogeneously mixing the Safeview into the agarose before pouring**.
  - *Result*: DNA might not be visible in all parts of the gel.

- **Over-cooling the agarose before pouring**.
  - *Result*: Safeview will not be evenly distributed in the gel, and/or the gel have lumps and not be of uniform thickness because some of the agarose will have already started polymerizing.
- **Running a gel with a lot of bubbles in it**.
  - *Result*: DNA migration can be impeded/distorted by bubbles in the gel. Usually as a gel is cooling, bubbles will migrate to the top where (usually) they will be out of the path of the DNA unless the DNA volume fills the wells to the top. But bubbles can sometimes occur deeper in the gel – particularly in a very high % agarose gel. Try to nudge bubbles out of the way before the gel solidifies, either by raking them with a comb that is not on a holder or nudging individual bubbles with a pipette tip before the gel congeals. If the gel is already solid, avoid loading in lanes that will run across a bubble.
- **Running a gel that wasn't mixed homogeneously before pouring in the mold.**
  - *Result*: there will be lumps in the gel of more dense agarose that will cause distortion of how the DNA migrates.
- **Accidentally contacting the agarose with a pipette tip**.
  - *Result*: the bottom or side of the well can become perforated, causing the sample to leak out the bottom or into the next well. If the tip is pressed against the agarose, the sample can be forcefully and suddenly expelled and blast out of the well.
- **Overloading the wells with too much sample volume**.
  - *Result*: samples can become cross-contaminated by DNA from adjacent wells.
- **Overloading the lane with too much DNA**.
  - *Result*: DNA can become retarded during running and the apparent size will not be accurately gauged by the ladder.
- **Forgetting to load a DNA ladder**.
  - *Result*: you won't know if your DNA bands are the right size when you look at and photograph your gel.
- **Not using gel loading mix in the DNA or using ladder that isn't premixed with gel loading mix**.

  - *Result*: The DNA or ladder (which is also DNA) will be lost mostly during loading without the gel loading mix that makes it sink to the bottom of the wells in the gel and stay there while you are loading. Both your samples and the ladder are DNA in an aqueous solution that is about the same density as the buffer (usually) – therefore they both should have gel loading mix in them in order to be denser than the tank buffer. The gel loading mix also includes convenient, charged dyes that migrate at different rates to help you see that your gel is running and how far it has progressed. The gel loading mix types only really matter if one type happens to have a dye that migrates exactly like your band of interest - in that case, you may want to switch to a different mix so that the dye doesn't block the fluorescence of your band during visualization.

- **Not thoroughly mixing gel loading mix into your DNA before loading**.
  - *Result*: you could lose part of your DNA to floating out of the well during loading. (This can also happen if there are other reasons your DNA is not very dense, such as if there is residual ethanol in it from preceding processing. In that case, you will see your DNA rapidly floating up to the surface as soon as you start expelling it from the tip).

- **Adding premixed DNA ladder to your samples instead of gel loading mix**.
  - *Result*: ladder will appear in every lane, obscuring your bands and wasting expensive ladder.

- **Forgetting to start the current on your loaded gel**.
  - *Result*: DNA will diffuse both out of the wells and into the gel in all directions causing loss of some of the DNA and blurriness of the remaining DNA once the gel is run. Diffusion can also happen if it takes a long time to load the gel. If there are a great number of samples to load – such as in a two- or three-comb gel, it is often best to load one tier and run the gel for 5 minutes so the DNA enters the gel and diffuses more slowly before moving on to load the next tier. Diffusion can happen more quickly if the buffer is warm from a previous run. If the buffer is warm, replace it before trying to load another gel in the same box.

- **Not keeping track of the order of sample loading**.
  - *Result*: you won't know which lane corresponds to which sample. This can happen, for example, when loading samples from a strip of PCR tubes, where their order can accidentally be rotated 180 degrees, or when loading from a PCR plate, where the plate orientation can be rotated 180 or 90 degrees (e.g., wells are loaded with respect to rows versus columns).

- **Running your gel backwards by accidentally reversing the positive and negative electrodes**.
  - *Result*, your DNA will migrate out of the end of the gel nearest the wells (the "top" of the gel) and be lost into the tank buffer. Always check that your electrodes are hooked up to the correct leads relative to the terminals at the power source, that your gel is oriented so that the DNA will run toward the positive pole. If you have already loaded your gel in the wrong orientation, do not lift the gel out of the tank, but just reverse the leads (red to black) to correct the current. Remember that DNA is negatively charged and will run to the positive (red) pole. When you start the current, the negative electrode (anode) at the well end of the gel should be making noticeably more bubbles than the positive electrode (cathode) at the bottom end of the gel. That's because electrolysis of water (H2O) will produce twice as much free hydrogen at the anode than the cathode makes free oxygen. Making a habit of checking that tells you two things: that current is really flowing and that you do have the right orientation of poles.
- **Running a gel at too low a voltage**.
  - *Result*: low molecular weight bands may look fuzzy and faint because they will be diffusing in random directions as they migrate.
- **Running DNA that is too salty - e.g., DNA in Phusion buffer or in NEB restriction buffer 3 (or 3.1) etc.**
  - *Result*: a "salt front" will form where DNA in those salty buffers will be slowed ("retarded") relative to the ladder, making the ladder a useless measure of the actual size of the DNA. Bands will be compressed at a "front" and will be a weird shape like a smile or a frown rather than a straight band.
  - If you know your DNA is in a salty buffer, you can remove this problem by cleaning the DNA first (i.e., via a cleanup column or with paramagnetic beads), or you can mitigate the problem by diluting only a few microliters (e.g. 5 µl) of your DNA into water and gel loading mix before loading (assuming you have a high enough concentration of DNA that it can still be seen if you load only a small fraction of it).
- **Running a gel made up in a different buffer (or different concentration of buffer) than the buffer in the gel tank; this includes using old buffer that has been evaporating in the tank through multiple runs or over time**.
  - *Result*: bands will not migrate as expected. "Fronts" may form where the leading edge of the migrating DNA is compressed.
- **Losing your gel of the end of the gel mold when you are taking it out of the tank**.

- *Result*: the gel could shatter and be unsalvageable. Solution: transport gels in a dish and be especially careful when moving flabby, low percentage agarose gels (under 1%).

B.3 Potentially dangerous and/or destructive mistakes:

> ⚠ Warning
>
> **Running gels is so standard in a laboratory that we can take if for granted, but these mistakes can be costly in terms of ruining a gel at best, or posing human safety risks at worst**

- **Heating a bottle with a lid on it**.
  - Result: possible explosion. Just leave the lid off when heating agarose.
- **Adding Safeview to molten agarose that is too hot**.
  - Result: much of the Safeview will be degraded by heat, causing your DNA to be hard to see or image. **The plastic gel mold could be permanently warped if the agarose is too hot.** The tape may fail, causing a mess in the fume hood and reagent wastage. A swirled bottle should be just comfortable (70 degrees C or a bit less).
- **Running a gel at too high a voltage**.
  - Result: **buffer could overheat during running and permanently warp the plastic of the gel box.** Bands might look smear and not be well resolved.
- **Dissolving agarose in water instead of electrophoresis buffer or putting water in the electrophoresis tank instead of buffer**.
  - Result - total failure for DNA to migrate into the gel, loss of samples. **If there is only water and not buffer in the tank, it could overheat and permanently warp the plastic of the gel box.**
- **Over-running your gel**.
  - Result: the DNA might run off the bottom of the gel and be lost. If you had more than one tier of wells in the gel, the DNA in the top tier will run into the zone of the next tier down, causing distortion and blurriness of the bands as they cross the wells of the lower tier and creating confusion in interpretation because very small fragments from the top tier will be overlapping very large fragments of the lower

tier. If the gel runs for a long time, the **buffer could overheat and permanently warp the plastic of the gel box.**

B.4 Conscietiousness

> ⚠ Warning
>
> We all work in our laboratory together. Please think of your labmates when you are done running your gel.

Please really clean the gel molds and combs after use. The next person shouldn't have to fish another lab mate's combs and molds out of the sink and clean and dry them before they can pour their gel. After pulling combs from a polymerized gel, make sure to really rub them under a flow of water; a skin of polymerized agarose left on the combs (higher percentages of agarose are especially prone to this) will mean the next person has to clean this off before they can pour a gel. Likewise, make sure the gel melting bottle is rinsed immediately after you pour your gel. If residual agarose is allowed to dry in the bottle, it can create an almost insoluble blob in the gel of the next person to use the bottle. If you see that some of your agarose has polymerized in the bottle, add water to the bottle and microwave it for a few minutes to dissolve and discard the residue.