

# Wildlife Genetics Lab

3-326A and 3-326B Warnell School of Forest Resources  
The University of Georgia  
Athens, GA 30602

## Policies and Information

# Welcome

Welcome to the Wildlife Genetics Lab of the Warnell School of Forest Resources. This document has been set up to acquaint you with some of the policies and procedures regarding use of the lab.

Please read this document in its entirety as you will be responsible for following the policies outlined within. The policies have been put together to keep everyone happy, productive, and efficient. Should you have questions about any of these guidelines or the protocols outlined herein, you may direct your questions to Brant Faircloth, the current coordinator of the lab.

In order to make this clear up front, you will be responsible for your own actions in the lab, and you are responsible for keeping an eye on others.

Violations of the guidelines provided herein will initially result in a warning. Subsequent violations of these guidelines may result in your expulsion from the lab. This is a necessary measure to ensure that no individual's work is jeopardized by actions of another. Please TAKE THIS SERIOUSLY!

Funding for this laboratory has generously been provided from several sources, including the University of Georgia, the Warnell School of Forest Resources, Tall Timbers Research Station, Quail Unlimited – Oconee County Chapter, the U.S. Department of Agriculture, and several private donors. It would be greatly appreciated if, upon completion of your molecular work, these entities be given credit in the "Acknowledgements" section of any manuscript you publish using data collected in this lab.

# Initial Requirements

Prior to beginning to work in the lab, you MUST have completed the “Right To Know” training provided by the University of Georgia. The training guide and documentation may be found here:

<http://www.esd.uga.edu/rtkcs/RTKChemSpec01.htm>

Upon completion of the training, you must print and sign the form provided. A copy of this form MUST be placed in the MSDS notebook that is in 3-326A prior to beginning any work in the lab.

Keep in mind that refresher training must be completed YEARLY.

Furthermore, prior to beginning work in the lab you must sign the form at the end of this document indicating that you understand and agree to abide by the guidelines outlined in the following pages.

Finally, welcome to the lab and good luck with your work!

# Contact Information

## Emergency Numbers:

Emergency: 9-911 (ACC Fire, Ambulance, Police)  
Campus Police: 2-2200

Environmental Safety: 2-5801  
Bio-Safety Office: 2-0112  
Poison Control: 9-800-282-5846  
St. Mary's Hospital: 9-548-7581  
Athens Regional Hospital: 9-549-9977

## Emergency Contact Information (call first available on list):

Brant Faircloth: 2-3932 (office)  
Lab Coordinator (706) 613-6963 (home)  
(706) 338-1349 (mobile)

John Carroll: 2-5815 (office)  
PI (706) 769-9635 (home)

# Lab Setup

The lab is setup such that it is composed of 2 rooms, named 3-326A and 3-326B. 3-326A is the room with a door directly into the hallway. It is the older of the two rooms, never having been remodeled. 3-326B is the newer of the rooms and houses the PCR and sequencing equipment.

## DNA Extraction :: 3-326A

3-326A has been setup as the pre-PCR lab. This means that ALL DNA extractions and work with tissues will be carried out in this lab. These activities are not to be carried out in the PCR lab. The primary reason for this is that extraction of DNA from samples increases the quantity of “free” DNA floating around in the lab. Since PCR is a very sensitive process, we want to minimize the amount of DNA floating around such that it does not find its way (inadvertently) into someone’s PCR reaction. The best way to minimize this risk is to separate PCR areas from non-PCR areas.

Extracting DNA in the 3-326B is a serious no-no. This would be an example of a flagrant mistake that jeopardizes the work of others.

## PCR :: 3-326B

3-326B has been setup as the PCR and sequencing room. It should contain all necessary items to carry out these tasks. DNA extraction should not occur in 3-326B, ever.

## Equipment

The following major equipment is available in the lab:

PE/ABI 377-96 semi-automated DNA sequencer, 2 Bio-Rad MyCycler 96-well thermal cyclers, 1 Bio-Rad MyCycler Gradient 96-well thermal cycler, Eppendorf 5804 benchtop centrifuge with 30-place and deep-well plate-rotors, chemical fume hood, 4 C refrigerator, GE -20 C chest freezer, Fisher Scientific 4C/-20 C Refrigerator/Freezer combination, Mettler-Toledo PL303 balance, regular and shaking water baths, and plates and cassettes for the PE/ABI 377-96 sequencer.

Other basic equipment including Rainin LTS pipets, a hotplate/stirrer, sample vortexers, a sample incubator, and necessary laboratory glassware are available in the lab.

## Other Laboratories (Nairn, Merkle, Covert)

There are several instruments or pieces of equipment that are available for use in other labs. These include a HYDRA microdispenser, autoclaves (2), a plate reading fluorimeter, milliQ and/or Nano-pure water systems, and dishwashers, among others.

When using these items, you WILL treat them as if they belonged to our lab, defer to anyone from other labs using them, and thoroughly clean the areas in which you did your work (even if the mess is not your own).

Furthermore, prior to beginning to use these instruments, you will find out from an appropriate person how the instrument operates and go through any necessary, instrument-specific training prior to using the instrument solo.

Failure to follow these simple rules also constitutes a serious no-no. This is especially true in others laboratories because your failure to act in an appropriate manner can hurt the rest of us who depend on these items to get our work done.

## Bench Space and Work Time

Given the limited size of the genetics lab, bench space and work time will both be at a premium. Additionally, there are several folks in the lab whose entire projects/dissertations/lives are based upon the work they are doing. Furthermore, most funds for the lab have been ponied up by their bosses.

Therefore, premium bench space has been reserved for these people. Other folks working in the lab will be given some bench space that is (perhaps) shared.

Given that there will likely be some sharing at some point, we will need to work out a schedule for those folks that are sharing such that we can get some work done.

Similar to signing up for sequencer/thermocycler time (see below) this will be handled via the web and a calendar client.

More details when this becomes an issue.

# Chemicals

There are several chemicals in the laboratory that may have serious effects if ingested or splashed in your eyes. The most potent among these are poly-acrylamide (neurotoxic) and Ethidium Bromide (teratogenic).

However, any chemical in the lab should be treated with respect. In 3-326A, the Material Safety Data Sheets (MSDS) are contained in a labeled notebook. These sheets outline all potential risks and effects of the chemicals in the lab, as well as detailing how to deal with spills and such.

You will look through these MSDSs to familiarize yourself with the information they contain.

All chemicals (primers excluded) that are ordered for use in the lab **MUST** be ordered using a standard purchase request form:

<http://www.forestry.uga.edu/warnell/network/forms/purchase.pdf>

and the “Ship to Address” should be listed as 3-326A. The University maintains a campus-wide database of chemicals and their locations, and this is the means used to ensure the chemicals are entered to the database.

3-326A should always be given as the ship-to, and the word “CHEMICAL” should be indicated somewhere on the purchase request form so Charis Lee can take the correct steps to get the stuff ordered.

Several stock solutions will be maintained in the lab for use by all projects. These will be indicated to you when you do your lab orientation.

Non-communal chemicals must be purchased by your project. In a pinch, you may borrow from others, but you **MUST** ask the owner of those chemicals prior to borrowing.

Borrowing of chemicals without authorization is another major no-no.

See Appendix A for a list of Common Solutions

# Primers

Ordering primers is a bit different than ordering “normal” chemicals. Given that primer sequences are often long, confusing, and easy to order incorrectly, the University has allowed labs to order their own primer sequences.

In order to do this, you must first create an account with two (2) different suppliers: IDT DNA and Applied Biosystems.

<http://www.idtdna.com> (HEX and 6-FAM labels)

<http://www.appliedbiosystems.com> (NED labels; also 1/2 quantity of IDT)

When setting up this account, first make sure you will be paying via purchase order (PO). Then, you are to put in your billing information:

ATTN: Charis Lee  
Warnell School of Forest Resources  
University of Georgia  
Athens, GA 30602  
TEL: (706) 542-4747  
FAX: (706) 542-8356

For shipping, put:

ATTN: {Your Name}  
3-326A Warnell School of Forest Resources  
University of Georgia  
Athens, GA 30602  
TEL: (706) 542-3932

To place an order (Brant will go over ordering process with you, or he will order your primers for you, using your new account), you must first go to Charis Lee and request a PO to either/both IDTDNA or Applied Biosystems. She will then give you the PO number (keep them straight!) which you will use when you set up your order.

If you order from IDT and have primers setup as such:

/HEX, 6-FAM/ - GCA AGG ... GCA

IDT will likely call you to inform you of a problem with Guanine quenching of fluorescent tags. Usually this is not a problem, and you can tell them to place the order. For more info, go here:

[http://www.idtdna.com/program/techbulletins/Quenching\\_by\\_Guanine\\_Base.asp](http://www.idtdna.com/program/techbulletins/Quenching_by_Guanine_Base.asp)

# Primers and PCR Reagents

Primers for each research group will be aliquoted from the stock solution into volumes sufficient for the analyses you are conducting (e.g. 100 ul aliquots for a 96-well plate when you use 1 ul per sample).

Brant will be doing the aliquoting of primers for each group (at least at first). This is to keep you from screwing yourself by contaminating the primers on which you spent major bucks.

Primers will be contained in an appropriate box/rack for your project and labeled. You should go into this box and this box only for your primers. Going into other people's/project's primers/reagents will not be tolerated.

Reagents will be handled in a similar fashion, at first. Once you become comfortable with this system and using the lab in general, you will make your own aliquots (with the exception of the quail folks, for whom I will be doing this).

I would **STRONGLY** advise you to set up these aliquots just as they were set up for you [in volumes sufficient for the number of individuals you are investigating in each group, e.g. 94 (plus + and – control)].

Things should work that way for a reason. In being this anal retentive about your primers and reagents, you keep yourself (hopefully) from contaminating all your stocks. If things get contaminated, it is generally only one set of aliquots that can be thrown away. Failure to do things this way will result in lost money and time, something none of us have in unlimited supplies.

Furthermore, Brant will help folks diagnose contamination problems **ONE** time and one time only. After that, it is up to you, especially if you have refused the advice above.

See Appendix B: PCR Protocol and Description for more information.

# Thermocyclers and Sequencer

The thermocyclers and the sequencer are the hallowed gods of our operation. That being said, before you use them, you need to know what you are doing and why. These are all VERY expensive machines that can be easily broken. Proper use of these machines should be covered in some detail during your lab orientation.

It is imperative that you do not mess with/harass/harm these pieces of equipment by doing something silly.

Therefore, until you have a full understanding of setting up either the thermocycler or the sequencer, someone with knowledge of what they are doing will help you accomplish these tasks (probably several times in the case of the sequencer).

With respect to the thermocyclers, we will set-up a program that contains all the necessary steps to run your PCR reaction. That way, when you need it in the future, you just choose the program, hit start, let the thermocycler warm up, put in your samples and let it run.

## Protocols for Sequencer and Thermocycler

See:

Appendix C: Preparing 12 inch Gel for ABI 377

Appendix D: Preparing Samples for ABI 377

Appendix E: Running 12 in. Gel on ABI 377

## Scheduling Sequencer and Thermocycler Time

Given that there will be numerous folks using these pieces of equipment, we will be scheduling time for each of them using a calendar program that links to the web. That way, you can see who is scheduled to operate what piece of equipment at what time, and you can find out when they will be done.

The calendar (read only) will be accessible at:

<http://gallus.forestry.uga.edu/lab/calendar/>

In order to make additions or changes to the calendar, you will be required to use the Mozilla Calendar extension to Mozilla Firebird or some other calendar program compatible with the .ics calendar format (Apple ical, etc.).

This will be covered in more detail later.

# Lab Policies

Instructions: fill out the required information requested by this form and give the copy to Brant Faircloth prior to beginning work in the Genetics laboratory. A copy of this form will be kept in “the book” should you need it for anything.

Name: \_\_\_\_\_

Project: \_\_\_\_\_

Degree: \_\_\_\_\_

(1) I have read the preceding guidelines regarding my use of the Wildlife Genetics lab and I agree to follow them.

(2) I understand that failure to follow these guidelines could lead to my expulsion from the lab following > 2 warnings for major infractions (these include but are not limited to: goofing around in the lab, breaking equipment, using others reagents, etc. w/o permission, and other negligent acts).

(3) I have provided my major professor with a copy of this information so that they will have knowledge of this agreement.

\_\_\_\_\_  
Your Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Maj. Prof. Signature

\_\_\_\_\_  
Date

## Appendix A

### List of common solutions

#### Blue-Dextran/EDTA Buffer (for preparing samples for 377)

25 mM EDTA  
50 mg/ml blue dextran

Also available as P/N 402055 from ABI

#### Digestion/Storage Buffer for Samples (Equivalent to Buff. ATL from Qiagen Kit)

1000 ml

100 ml 1.0M Tris  
200 ml 0.5M EDTA  
2 ml 5.0M NaCl  
100 ml 10% SDS  
598 ml H<sub>2</sub>O

#### DTT Solution (for feather digestion)

Add 0.100 g DTT (Dithiothreitol) to 1 ml dH<sub>2</sub>O in 1.5 ml microtube.  
Vortex until dissolved.

#### 0.5 M EDTA

For 500 ml:

EDTA will not dissolve unless the solution has a pH of 8.0

Dissolve about 7 g of NaOH pellets in ~300 ml of dH<sub>2</sub>O, then add 93.1g Na<sub>2</sub>EDTA (make sure you are using **disodium** salt and not a different one). Continue adding NaOH pellets until the pH is about 8.0. Now the EDTA will dissolve (it is fairly slow!). Bring volume close to 500 ml, then adjust the pH to 8.0 with 10M NaOH. Adjust the final volume to 400 ml, double-check the pH and adjust as needed.

EtBr Solution

Add 5mg/ml to dH<sub>2</sub>O

Loading Buffer for PCR Product

	<u>1X</u>	<u>100X</u>
De-ionized formamide	3.0 ul	300 ul
Dextran/EDTA Buffer	1.2 ul	120 ul
ROX 500	0.3 ul	30 ul

40% 19:1 Polyacrylamide Mixture

!!NOTE!! : Do NOT make this yourself unless you have prior experience making this solution. Required PPEs (Personal Protective Equipment) for making this solution are (1) lab coat, (2) gloves, (3) safety goggles/glasses, (4) Mask-style particulate respirator. Polyacrylamide is a neurotoxin, and the powder may become airborne very easily, potentially entering the lungs. This is bad.

	<u>25ml</u>	<u>50ml</u>
19:1 Bis-Acrylamide powder	10g	20g
MilliQ (ultra-pure) ddH <sub>2</sub> O	15ml*	30ml*

In a beaker, combine the powder and the MilliQ water and stir with heat (set plate at 80 C) until solute dissolves (5 minutes or so). Pour solution into an appropriately sized graduated cylinder and bring solution up to the total volume using MilliQ ddH<sub>2</sub>O. Place solution into an amber 250ml container and put in the refrigerator.

If you feel strangely following the mixing of the solution, do not delay in going to the emergency room. Make sure to carry the bottle (or note its label) with you when you go.

NOTE: You must use MilliQ or equivalent grade water for this solution. Use of deionized water will result in gel solution that is not adequate for fragment sizing, and your results (and perhaps those of others) will suffer.

## 1 M Tris

For 1 L:

**Caution:** There is Tris-base (MW=121.1) and Tris-Hcl (MW=157.6). Be sure you get the correct one!

Dissolve 121.1 g Tris-base in 700 ml of dH<sub>2</sub>O, then adjust to pH 8.0 using concentrated HCl (~15 ml). Dilute to 1000ml with dH<sub>2</sub>O, and double check the pH. Adjust as needed with HCl.

## TE (Tris-EDTA; 10mM Tris, 1.0 mM EDTA)

For 1 L:

10.0 ml Tris, pH=8.0  
2.0 ml 0.5 M EDTA

Bring final volume to 1000 ml with dH<sub>2</sub>O

## TLE (Tris-Low EDTA; 10 mM Tris, 0.1 mM EDTA)

For 1 L:

10 ml Tris, pH=8.0  
200 ul 0.5 M EDTA

Bring final volume to 1000 ml with dH<sub>2</sub>O

For 250 ml:

2.5 ml Tris, pH=8.0  
50 ul 0.5 M EDTA

## 10X TBE (Tris-Borate-EDTA)

For 0.5 L:

54.0 g Tris-base  
27.5 g Boric Acid  
4.15 g EDTA (make sure it is **disodium** salt)  
500 ml dH<sub>2</sub>O (MilliQ)

For 1 L:

108 g Tris-base  
55 g Boric Acid  
8.3 g EDTA (make sure it is **disodium** salt)  
1000 ml dH<sub>2</sub>O (MilliQ)

Try and use within 7-10 days.

# Appendix B

## DNA Extraction Protocol For Feather Samples<sup>1,2,3,4</sup>

- 1) Prepare 100 mg/ml DTT solution.

Measure 0.100 g of DTT and add to 1 ml water in 1.5 ml microtube. Shake or vortex to dissolve.

Discard DTT solution after use as it does not do well in storage.

- 2) Place up to 25 mg tissue in 1.5 ml microtube, and add 180 ul **Buffer ATL**.
- 3) Add 20 ul **Proteinase K** and 25 ul **DTT** solution. Mix by vortexing for ~15 sec. Incubate @ 55 °C in shaking water bath (or equivalent) until tissue is completely lysed.

Lysis is usually complete in 2-3 h, but may be carried out overnight.

- 4) Remove samples from water bath. Vortex for 15 sec. Add 200 ul **Buffer AL** to sample and vortex for 30 sec. Incubate at 70 °C for 10 min.
- 5) Add 200 ul **ETOH** (96-100%) to sample, and mix by vortexing. White precipitate may form with addition of ETOH. Be sure to add all precipitate to spin-column.
- 6) Place **Buffer AE** into 55 degree water bath (heating the elution buffer will increase DNA yields from the membrane).
- 7) Pipet mixture from step 5 into DNeasy spin column sitting in 2 ml collection tube (provided). Centrifuge at 8000 rpm for 1 min. Discard flow-through and collection tube.
- 8) Place DNeasy mini-column in a new 2 ml collection tube (provided), add 500 ul Buffer AW1, and centrifuge at 8000 rpm for 1 min. Discard flow through and collection tube.

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<sup>1</sup> If you are digesting tissue with the Qiagen Kit, follow protocol given in kit.

<sup>2</sup> If you are digesting mammal/bird blood samples, think about using Amersham Biosciences Genomic Prep Blood DNA Isolation Kit (Qiagen Kits may be used, however).

<sup>3</sup> If you are digesting feather shafts, follow the protocol given

<sup>4</sup> Modified from Qiagen Inc.'s DNeasy kit protocol.

- 9) Place DNeasy mini-column in a 2 ml collection tube (provided), add 500 ul **Buffer AW2**, and centrifuge at 10,000 rpm for 3 min

Be careful that spin-column does not contact the flow-through in this step (leading to carryover of ETOH). If this happens, discard flow through and centrifuge again at 8000 rpm for 1 min.

- 10) While these sample are spinning, remove the tops from a number of microtubes equivalent to the number of samples you are processing.
- 11) Go get **Buffer AE** from the water bath.
- 12) Place DNeasy column in clean 1.5 ml microtube with top removed. Pipet 60-80 ul **Buffer AE** directly onto membrane (for adult feathers or patagial tissue). Incubate at room temp. for 1 min. Centrifuge at 10,000 rpm for 1 min to elute.

Close tube, label, and store. If needed, carry out second elution using clean 1.5 ml microtube and 60 ul Buffer AE as above. Close tube, label and store.

## Appendix C

# PCR Protocol and Description

The Polymerase Chain Reaction (PCR) is the primary means we use to amplify DNA to quantities that we can subsequently use for fragment analysis (among other things).

PCR is an interesting and fickle beast requiring the amalgamation of several reagents (the reaction “cocktail”) used in combination with electronically-controlled fluctuations in temperature (via a thermocycler) to produce pieces of DNA region of interest which lies between the 2 primers we chose to use [these are denoted in this lab as the “Upper” (“Forward” in other labs) and “Lower” (“Reverse”) primers].

PCR was “discovered” by Kary B. Mullis of Cetus Pharmaceuticals in 1983 while he was driving along a roadway in Mendocino County, CA. In 1992, Hoffman-LaRoche purchase the rights to use *Taq* polymerase in the PCR reaction, and their purchase supposedly included all subsequent licenses for the commercial production of *Taq*. However, their ownership of right to use *Taq* in PCR (and the PCR process, in general) has been challenged in court after Hoffman-LaRoche filed suit against Promega Corp.

Anyway, PCR reactions require 7 essential components [following Sambrook and Russell (2000)<sup>5</sup> ]:

### 1) Thermostable DNA Polymerase:

The polymerase allows the catalyzation of template-dependent synthesis of DNA . Typically, *Taq* polymerase (isolated from *Thermus aquaticus* bacteria) is the chosen thermally stable polymerase, but others exist such as *Pfu* polymerase. Most PCR reactions will contain  $2 \times 10^{12}$  to  $10 \times 10^{12}$  molecules of *Taq*.

### 2) Synthetically derived oligonucleotides (oligos/primers) to prime the synthesis of DNA

The oligos anneal to specific locations on each strand of DNA (upper or lower) allowing amplification to occur between their binding regions. Typically, 0.1 to 0.5 M of each oligo are included in the PCR reaction. High concentrations of primer may lead to mispriming and spurious peaks. Oligos may be modified in a number of ways, including the addition of 5' or 3' fluorophores allowing scoring on an automated sequencer.

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<sup>5</sup> Sambrook, J. and D. Russell. 2000. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 2344p.

3) dNTPs (Deoxynucleoside triphosphates)

dNTPs are the bases that will be integrated to the strand(s) of DNA being built by the PCR process. Obviously, there are 4 dNTPs: dTTP, dCTP, dATP, dGTP. Typically, 200 to 250  $\mu$ M of each dNTP are used in a reaction. High dNTP concentration may lead to inhibition of PCR reactions by sequestering  $MgCl_2$ . dNTPs may also be fluorescently labeled (like primers) to allow fragment sizing using automated DNA sequencer.

4) Divalent Cations

Thermostable DNA polymerase requires free divalent cations to work properly. Therefore,  $MgCl_2$  is typically added to reactions. Both dNTPs and oligos can bind  $Mg^{2+}$ , and the concentration of  $MgCl_2$  required for a reaction varies, being dependent upon the concentration of phosphate groups contributed by dNTPs plus oligos. A concentration of 1.5 mM  $Mg^{2+}$  is typical.

5) pH Maintaining Buffer

Tris-Cl is typically included to help buffer the pH of reactions. Tris is typically included along with the following ingredient (and perhaps some others) in the "reaction buffers" provided by supply companies (AbGene, Sigma, Promega, etc.).

6) Monovalent Cations

50 mM KCl is also typically included as a component of PCR buffers.

7) Template DNA

Certainly a necessary ingredient! PCR technically only requires one copy of the template sequence, but numerous copies (e.g.  $\sim 3 \times 10^5$ ) are typically added to the reaction.

A typical PCR reaction is run at a total volume of 25 or 50  $\mu$ l. However, we are cheap and will try and run reactions at 10  $\mu$ l. Provided all goes well at this volume, it is cheapest and easiest to remain there. However, if things do not work out at 10, we can always go to 50  $\mu$ l.

A typical reaction mix for a single sample is detailed below – parentheses given concentrations in reaction mix, not concentration of stock:

Sigma Jump Start Taq (0.25U):	0.1 l
Reaction Buffer (1X):	1.0 l
Magnesium Chloride (1.5mM):	0.6 l
dNTPs (2.0mM of each base):	1.0 l
Upper Primer (1mM):	1.0 l
Lower Primer (1mM):	1.0 l
Double distilled water (ddH <sub>2</sub> O):	3.3 l
Template DNA:	<u>x.x l</u>
 Final volume:	 <b>10 l</b>

Typically 2.0 l of template would be added and the reaction would be brought up to volume with ddH<sub>2</sub>O (3.3 l), a sealing mat or strip-tube top would be applied, and the reaction would be run on the cycler. If you want to use more or less template DNA, you need to adjust ddH<sub>2</sub>O accordingly.

When setting up reactions, you typically do not want to set up individual reactions for each sample. Typically, reactions are set-up according your needs. In the lab, this will typically involve the use of 96-well plates (hence, setting up reactions for 94 samples with a + and – control). However, due to pipetting error, we tend to make up “master mix” for more samples than we plan to run. So, assuming we are using Eppendorf Pipets (which have a lot of error) and 2.0 l of template per sample, a “master mix” for 102 samples would look like this:

Sigma Jump Start Taq (0.25U):	10.20 l
Reaction Buffer (1X):	102.0 l
Magnesium Chloride (1.5mM):	61.20 l
dNTPs (2.0mM of each base):	102.0 l
Upper Primer (1mM):	102.0 l
Lower Primer (1mM):	102.0 l
Double distilled water (ddH <sub>2</sub> O):	336.6 l
Template DNA:	<u>x.x l</u>
 Final volume:	 <b>1020 l</b>

We would pipet 8.0 l into each well of the 96-well plate and then add our 2 l template DNA to each well.

# Appendix D

## Preparing 12 inch gel for ABI 377

Polyacrylamide Gel Solution (4.8% Gel)

\*Instructions are for preparing gel solution and casting a 12 in. well-to-read gel for the ABI 377 DNA Sequencer.

- 1) Make sure plates are clean and smudge-free. If necessary, wash again with lab soap and dH<sub>2</sub>O and allow to air dry. Plates may be dried using larger Kimwipes.

Rinse spacers, comb and casting device with dH<sub>2</sub>O as well and allow to air dry.

- 2) Prepare 10X TBE buffer, if necessary, and use within 7-10 days:

For 0.5 L: 54.0 g Tris, 27.5 g boric acid, 4.15 g EDTA in 0.5 L dH<sub>2</sub>O

For 1.0 L: 108 g Tris, 55 g boric acid, 8.3 g EDTA

- 3) Add first 4 ingredients listed below together in a 150 ml or 250 ml beaker. Mix with a stir-bar on medium speed for 5 minutes.

A) Urea	5.4 g
B) 40% (19:1) Acryl/Bis	1.8 ml
C) dH <sub>2</sub> O	9.0 ml
D) Amberlite Resin (AG501X8)	0.3 g
<hr style="width: 50%; margin-left: 0;"/>	
E) 10X TBE	1.5 ml
F) APS (100 mg/ml)	120 $\mu$ l
G) TEMED	12 $\mu$ l

Total Volume 15 ml

- 4) Set up filtering vacuum flask with 0.2  $\mu$ m filter and FIRST filter 10X TBE, then the acrylamide solution. Do not filter the TBE after the acrylamide solution--the Amberlite will remove the ions from the TBE (this is bad).
- 5) Degas filtered solution for 5 minutes.
- 6) While degassing solution, make sure plates and associated items are clean. Wipe face of plates off with Kimwipes if necessary.

- 7) Place cassette on top of Styrofoam blocks to raise it off working surface, Put some paper towels at bottom and top edge to soak up any leaky acrylamide. Place rear plate into the cassette (ETCHED SERIAL NUMBER DOWN), and slide forward until plate “locks” into place in cassette. Take spacers and moisten one side of each (opposite sides of spacers as they should face each other with the same orientation: namely skinny part at top of rear plate and grooves pointed towards each other).

Stick spacers down onto rear plate and make sure there are no air-pockets between plate and spacers. Allow spacers to dry onto plate.

- 8) Place front plate on the top end of the rear plate, making sure that the space for the comb is oriented to the inside of the two plates (to allow comb to be placed in its home).
- 9) Get acrylamide solution. Get ready to add APS and TEMED to solution and then finish the rest of the process ASAP so gel does not polymerize while you are still futzing around with it.
- 10) Method 1: Draw gel solution (after adding APS and TEMED) into syringe. Put the rear edge of the front plate in your other hand. Squirt (gently) gel solution under edge of front plate and gently move front plate so that it is resting on top of rear plate. Begin sliding front plate towards the bottom of the cassette while squirting acrylamide solution in front of plate edge. Back off if any bubbles form and resume when they are dislodged.

Method 2: Put rear edge of front plate in one hand. Gently move front plate so that it is resting on top of rear plate. Gently pour contents of beaker containing acrylamide onto the back plate in front of the edge of the front plate. Slide the front plate towards the bottom of the cassette while still pouring acrylamide solution. Back off if any bubbles form and resume when they are dislodged.

Slide front plate all the way down so that the bottom edges of the two plates are flush. Lock plates in place and place comb (shark’s-tooth side pointing out – e.g. don’t stick shark’s tooth part into gel) into gel.

- 11) Place 3 “Bulldog” clamps across top of gel (equally spaced) and make sure that they do not contact the comb.
- 12) Allow 1.5 to 2 hours for gel to dry prior to use. If preparing for a morning run, wrap bottom end of plates in Saran Wrap so that gel does not crystallize.

Additional Gel Concentrations

## 5.6% Polyacrylamide Gel

A) Urea	5.4 g
B) 40% (19:1) Acryl/Bis	2.1ml
C) dH <sub>2</sub> O	8.7ml
D) Amberlite Resin (AG501X8)	0.3 g

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E) 10X TBE	1.5ml
F) APS (100 mg/ml)	120 l
G) TEMED	12 l

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Total Volume 15 ml

## 6.0% Polyacrylamide Gel

A) Urea	5.4 g
B) 40% (19:1) Acryl/Bis	2.25ml
C) dH <sub>2</sub> O	8.55ml
D) Amberlite Resin (AG501X8)	0.3 g

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E) 10X TBE	1.5 ml
F) APS (100 mg/ml)	120 l
G) TEMED	12 l

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Total Volume 15 ml

# Appendix E

## Preparing samples for ABI 377

- 1) Prepare loading mix for PCR product

	<u>1X Conc.</u>	<u>100X Conc.</u>
A) De-ionized formamide	3.0 l	300 l
B) 377 loading buffer	1.2 l	120 l
C) ROX 500	<u>0.3 l</u>	<u>30 l</u>
Total	4.5 l	450 l

- 2) Load mix (4.5 l per well) into 96-well plate (clean).
- 3) Make sure all 96 well plates (containing product) are spun down prior to pipetting PCR product from plates. Otherwise, the pipettor will be VERY inconsistent.
- 4) Prepare to load PCR product into wells. This is marker dependent, but a good place to start is 1 ul per primer\*.
- 5) When multiplexing (adding multiple dyes to one lane), add the same quantities of the above to each well of the 96-well plate containing the master mix described above. THOROUGHLY mix samples by pipetting up and down within the well when the last PCR product is added.
- 6) Cover plate with clear 96-well seal. Spin down now or prior to denaturing and loading sample.

\* This is tricky for the following reasons: (1) when pipetting volumes lower than about 1 l, pipettor error can cause a serious problem for you when you pipet various volumes ranging from 0.4 to 0.6 l into the 377 load. This results in some bands that are ok, some that are not bright enough and some that are too bright. The 1:11 dilution is problematic because mixing the sample in the ddH<sub>2</sub>O does not typically go very well. This is why a shift to a smaller, more manageable volume of water might be better.

# Appendix F

## Running 12 in. Gel on ABI 377

After preparing gel (see Appendix B)....carry it to the 377 room.

- 1) If sequencer is off, turn sequencer on and start-up or reboot the computer. If sequencer is already on, reboot the computer before proceeding.
- 2) Make sure glass around the laser is as clean as it can get. Use kimwipes and H<sub>2</sub>O to clean it to the greatest degree possible.
- 3) Go ahead and remove comb from gel. Carry gel from prep area over to 377.
- 4) Check to see if bottom tray is clean, if not, empty and rinse it out and replace.
- 5) Choose FILE-> NEW
- 6) Choose Pre-run module GS PR 12D-2400
- 7) Choose Run module GS Run 12D-2400
- 8) Choose the **matrix** fitting to your project, **Well to read distance=12, Collect Time=2.0 (hours)**
- 9) Now, run plate check. To see what is going on choose  
WINDOW->STATUS  
  
If you see large spikes in the viewing area of the window...you need to re-clean glass.
- 10) To re-clean glass, choose INSTRUMENT->CANCEL RUN. Re-clean plates.
- 11) Run plate check again and check for spikes in output.
- 12) If no spikes, go ahead and proceed. If spikes, clean plates again, run plate check again.
- 13) Prepare 1500 ml 1X TBE (150 ml 10X TBE + 1350 dH<sub>2</sub>O).

- 14) Carry TBE and top tray over to machine. Add TBE to top tray so that it just flows over the lip of the comb area. Add TBE to bottom tray so that it just spills into catchment device on the front of the tray.
- 15) Grab syringe and dye, fill syringe w/ 5 ml or so of dye and run across lip of area where comb was. Check to see if all of dye moves between plates. Run pipet tip across area to free it of any acrylamide that may be in the wells. Once this is done, go ahead and insert sharks-tooth comb into well area until teeth reach the bottom of the well (do not insert into gel).
- 16) Go to PAPER beside RUN MODULE, click it, and make sure VOLTAGE=750, CURRENT=60, WATTS=200.
- 17) Now, choose PRE-RUN, click on WINDOWN->STATUS to see what is going on.
- 18) While gel is heating up, choose NEW->GENE SCAN SAMPLE SHEET, and fill out information for each well as it will be entered.
- 19) Once Gel temperature, as indicated by STATUS window, reaches 45 C, spin 96-well plate down and put it on the heating block for 5 minutes at 95 C. Remove samples after 5 minutes, put immediately on ice, and get ready to load.
- 20) Hit PAUSE to pause pre-run.
- 21) Load odd-numbered samples in odd-numbered wells. Flush multi-channel loader dH<sub>2</sub>O. Make sure loader is not in open position and draw 2 l of sample into loader. Hold loader over paper towel and push out enough sample to see it on the tips of the loader. Blot on paper towel. . Open loader to load lanes. Close after loading lane. Flush again. Continue loading lanes until all odd-numbered lanes are loaded.  
  
**If loader will not easily open and close, DO NOT FORCE IT. See Brant and he will show you how to fix the problem.**
- 22) Run gel for 2 minutes. After 2 minutes, PAUSE run and load even-numbered samples. When you are finished loading the gel, flush the loader with dH<sub>2</sub>O and rinse bottom of loader with dH<sub>2</sub>O if you have accidentally placed it in the TBE buffer.
- 23) TERMINATE PRE-RUN and begin RUN.
- 24) Run will complete in the amount of time you specified (2 hours). However, if you see something wrong, you can cancel the run, remove the plates and start over. Similarly, if the fragments you are sizing are generally

small, you can terminate the run after you see all labeled fragments have passed the laser AND there are >2 bands of size standard following your bands.

- 25) After finishing/canceling a run, remove the upper and lower basins, rinse them with dH<sub>2</sub>O, and set them out to dry. Get some windex and spray it on a paper towel. Then wipe down the white area of the sequencer against which the plates were sitting. Clean up any other spills/mess.
- 26) Clean your plates! This is easiest if done immediately after your run has completed. While wearing gloves, run hot water over the surface of the plates in the sink. You will feel the acrylamide begin to soften. You should now be able to twist the plates against one another (do this under the water), and they will eventually come apart. Rinse off all acrylamide, put A LITTLE bit of Liquinox on the plate and wash with a sponge. Make sure you rinse with dH<sub>2</sub>O after washing – or you will get spots. Rinse of the comb and spacers, wash with Liquinox, and rinse with dH<sub>2</sub>O. Let all sit out to dry.