

*Supplemental Material for –*

## **DNA Fingerprint Analysis of Three Short Tandem Repeat STR Loci for Biochemistry and Forensics Laboratory Courses**

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### **Index for this document** (*Supplemental Material*)

A.	Instructions for the Student Lab report for PCR and STR analysis.	page 2
B.	EXPERIMENTAL PROCEDURES	
	Step I: Isolation of DNA using the Promega DNA IQ™ System	page 3
	Step II: PCR Amplification of 3 STR Loci using Promega's <i>Geneprint</i> ® STR Systems	page 5
	Step III: Polyacrylamide Gel Electrophoresis for visualizing the 3 PCR Amplified STR Loci Products using Promega's Silver Stain Detection	page 8
C.	A description of Hardy-Weinberg population genetics and its significance in DNA fingerprint analysis (plus an example calculation of allelic frequency)	page 11
D.	Supplemental Figure 1 and Figure 2	page 14
E.	Important Points when Preparing Large Acrylamide Gels and twelve electronic images of large polyacrylamide gel setup and pouring.	page 15

## A. Instructions for the Student Lab report for PCR and STR analysis.

1. Contamination is a serious issue in police labs, and great care is taken to avoid contaminating samples. List at least 5 things you could do to avoid contamination which we were not necessarily able to do because of the constraints of performing this analysis in an undergraduate biochemistry lab. Discuss how you might set up controls to check for the possibility of contamination.
2. Analyze your PCR gel and describe the banding pattern you observe. Discuss the differences between the separation of DNA on an agarose gel versus an acrylamide gel. Include a description of supercoiled/nicked and linear DNA.
3. A criminal left blood at a crime scene which was analyzed at 3 loci in the forensic biology lab and compared with your DNA. What is the probability that your DNA will match that found at the “crime scene”? For heterozygous loci the formula  $P = 2pq$  is used where  $P$  is equal to genotype frequency for that loci, and  $p$  and  $q$  are the frequencies of each allele in the general population. When an allele is heterozygous why is the product of  $p$  and  $q$  multiplied by a factor of 2? Why is the relationship  $P = p^2$  used for homozygous loci? If you have an allele which is listed as 0 in the frequency table, the standard method is to use a minimum of 0.002 as the frequency for that allele. This is done so that a rare allele does not artificially inflate the probability. For independent loci the genotype frequencies can be combined through multiplication. The product rule is used to obtain the complete profile probability and is equal to the probability determined for each loci multiplied together (*i.e.*, probability of a match =  $P_1 \times P_2 \times P_3$ ). The proper way to state the resulting probability is that there is a  $1/(P_1 \times P_2 \times P_3)$  chance that some other person would randomly match that particular combination of alleles at all three loci.
4. In addition to the above analysis, please provide comments on the different aspects of this laboratory exercise. For example, what part of the instructions in the protocol package could be clearer? What part of this experiment do you believe needs to be improved? Were there any parts of the lectures that you didn't understand?

## B. EXPERIMENTAL PROCEDURES

### Step I: Isolation of DNA using the Promega DNA IQ™ System

Adapted from Promega's DNA IQ™ System-Small Sample Casework Protocol – Technical Bulletin No. 296.

#### Solutions and Materials needed

DNA Binding resin (supplied in kit)  
Lysis buffer (supplied in kit)  
2X wash buffer (partially supplied in kit - Prior to the lab the teaching assistant will add 15 ml of 95% ethanol and 15 ml of isopropyl alcohol to the 30 ml bottle of the 2X wash buffer. The final concentration will then be 1X wash buffer.)  
Elution buffer (supplied in kit)  
95% Ethanol (user supplied)  
1 M DTT (user supplied)  
Isopropyl alcohol (user supplied)  
Vortexer (user supplied)  
95° C bath or heat block (user supplied)  
65° C bath or heat block (user supplied)  
Sterile foam tipped applicators (user supplied buccal swabs - Puritan Sterile Foam Tipped Applicators - REF 25-1506 1PF)

1. Preparation of lysis buffer: For every sample you will isolate, pipette 350  $\mu$ L of lysis buffer into a 1.5 ml microcentrifuge tube USING A NEW AUTOCLAVED PIPETTE TIP. Add 3.5  $\mu$ L of 1 M DTT to each 350  $\mu$ L sample buffer, and mix by inversion.
2. Remove foam buccal swab from sterile wrapping. While holding firmly, move vigorously along inside of cheek to collect buccal cells. Swab with both sides of the foam pad.
3. Place foam pad just inside a new 1.5 ml microcentrifuge tube, and apply pressure to snap off the plastic stem just above the foam pad.
4. Add 250  $\mu$ L of prepared lysis buffer and incubate at 95° C for 30 minutes.
5. Remove the tube from 95° C and vortex briefly.
6. Transfer the sample/lysis buffer to a new microcentrifuge tube and then use a 1 ml pipette tip to squeeze excess liquid from the foam pad by pressing it against the side of the tube. Add any recovered liquid to the sample/lysis buffer in the new tube. Briefly spin the microcentrifuge tube containing the foam pad in a microcentrifuge, and immediately collect any liquid in the bottom of the tube and add it to the same new microcentrifuge tube. Failure to collect the liquid at the bottom of the tube quickly will result in the liquid being soaked up again into the foam pad.
7. Vortex stock resin bottle for 10 seconds until resin is thoroughly mixed. USING A NEW AUTOCLAVED PIPETTE TIP remove 7  $\mu$ L of the resin and add it to the approximately 250  $\mu$ L of prepared lysis buffer which now contains your DNA. Make sure to keep the resin suspended until it is time to mix it with the sample, as it is heavy and will settle quickly.

8. Vortex the sample/lysis buffer/resin for 5 seconds and place the tube in the magnetic stand. Separation will occur instantly. If resin does not form a distinct pellet on the side of the wall, vortex again and quickly place sample back in the stand.
9. Carefully remove and discard all of the solution without disturbing the resin on the side of the tube. Note: if some resin is drawn up in the tip, gently expel resin back into tube to allow it to separate again by repeating step 8.
10. Add the remaining 100  $\mu$ L of prepared lysis buffer to the tube containing the resin. Remove the tube from the magnetic stand and vortex for 2 seconds at high speed.
11. Return the tube to the magnetic stand and collect and discard all lysis buffer.
12. Add 100  $\mu$ L of 1X wash buffer. Remove tube from the magnetic stand and vortex for 2 seconds at high speed.
13. Return the tube to the magnetic stand and discard all wash buffer.
14. Repeat steps 12 and 13 two more times for a total of 3 washes. Make sure that all of the solution has been removed after the last wash.
15. With lid open, air dry the resin in the magnetic stand for 5 minutes. Do not air dry for more than 20 minutes as this may inhibit removal of the DNA.
16. Add 100  $\mu$ L of elution buffer, close the lid, and vortex at high speed for 2 seconds. Place tube at 65° C for 5 minutes.
17. Remove the tube from 65° C, vortex 2 seconds at high speed, and immediately place in the magnetic stand. Tubes must remain hot until placed in the magnetic stand or yield will decrease.
18. Remove the elution fraction containing DNA and place in a new tube and store at -20° C.
19. The isolated genomic DNA is now ready for PCR amplification in the next step.

## Step II: PCR Amplification of 3 STR Loci using Promega's *Geneprint*® STR Systems

Adapted from Promega's *Geneprint*® STR Systems (Silver Stain Detection) – Technical Manual No. D004.

### Solutions and Materials needed:

Geneprint CTT multiplex primers	(supplied in kit)
STR 10X buffer	(supplied in kit)
K562 High MW DNA (10 ng/μL)	(supplied in kit)
Sterile water	(user supplied)
Taq DNA Polymerase	(user supplied)
Allelic ladder mix	(supplied in kit)
PCR Thermal Cycler	(user supplied)

### A. Preparation of the PCR Mixture:

This step must be performed on ice to insure the elimination of unwanted mispriming. Wear gloves to avoid any contamination and add the following reagents in the order listed to a sterile 0.2 ml eppendorf tube (final volume at the end of step A will be 25 μl).

1. For the genomic DNA purified from buccal swabs (described above) add the following components in the listed volumes -

<u>Component</u>	<u>Volume</u>
Sterile water	16.50 μL
STR 10X buffer	2.50 μL
CTT multiplex primer pair mix	2.50 μL
Taq DNA Polymerase	1.0 μL
	<hr/>
	22.5 μL      total volume

2. Pipette 2.5 μL of the previously isolated genomic DNA into the tube containing 22.5 μL of PCR mix.

### B. Preparation of the Positive Control Reaction (Known Template – K562):

The control reaction is prepared by adding all reagents in the same manner as that described in step-A (above). The only difference is that 2.5 μL of K562 DNA template is added to the tube instead of the previously isolated genomic DNA.

### C. Preparation of the Negative Control:

The negative control is prepared by adding all reagents in the same manner as that described in step-A (above). The only difference is that **NO** DNA template is added to the reaction tube. Instead by 2.5 μL of sterile DDi-water is added to the reaction tube so as to maintain the same volume as the previous two reaction tubes.

### D. The Temperature Cycle used for PCR Amplification of the 3 STR Loci:

Place all the tubes in the PCR Thermal Cycler, and begin the preprogrammed cycles listed below (please refer to Amplification Protocols 7 or 8, depending on available PCR Thermal Cycler).

1. 94° C for 60 seconds  
60° C for 60 seconds  
70° C for 90 seconds

Repeat the above cycle 10 times

2. 90° C for 60 seconds  
60° C for 60 seconds  
70° C for 90 seconds

Repeat the above cycle 20 times

3. 60° C for 30 minutes to finish elongation of all strands.
4. Hold at 4° C until the next morning. Then, store in refrigerator.

#### E. Preparation of samples for gel electrophoresis:

1. When the agarose gel has fully polymerized and is ready to run (following step - F), pipette 10 µl of the above PCR reaction mixtures into a fresh 1.5 µL microcentrifuge tube.
2. Add 3 µl of non-denaturing agarose gel loading buffer (0.25% bromophenol blue, 40% sucrose) to each of the above tubes.
3. The teaching assistant should prepare markers to run alongside the samples.

#### F. Agarose gel electrophoresis of DNA and PCR amplification products:

Agarose gel electrophoresis is used as a relatively rapid means of confirming the success (or failure) of the PCR amplification before performing the larger scale (and higher resolution) polyacrylamide gel electrophoresis analysis.

##### Solutions and Materials needed:

agarose gel running apparatus and power supply	(user supplied)
10X TAE buffer	(user supplied)
agarose	(user supplied)
ethidium bromide stock solution (1 mg/mL)	(user supplied)
molecular weight size markers (50 bp and 100 bp ladders)	(user supplied)

##### Procedure:

1. Make 1 L of 1 X TAE by adding 100 mL of 10 X TAE to 900 mL of distilled water.
2. Prepare a 2% agarose gel by adding 2.0 g of agarose to 100 ml of 1 X TAE buffer.
3. Heat until boiling, then remove and cool to 55° C before pouring into the gel tray.
4. While the TAE/agarose cools, apply tape/rubber stoppers to the end of the trays and position the combs.
5. Add 10 µL of ethidium bromide to the agarose solution (after it cools but before you pour it into a gel tray).
6. Pour the agarose into the tray and let cool until opaque, at this point it will be solid.
7. Prepare the samples by mixing the amplified STR's with the dye as specified in step E above.
8. Gently remove rubber stoppers and comb from polymerized gel.
9. Place the gel and tray into the electrophoresis gel box, pour the running buffer into the tank over the gel. The buffer should cover the gel but not run out of the electrophoresis unit. Gently remove the comb.

10. While wearing fresh gloves (*i.e.*, no holes) carefully add 6 drops (~10  $\mu$ L) of concentrated ethidium bromide solution (1 mg/ml) to the positive end of the gel apparatus, mixing it in well with TAE buffer.
11. Load each sample into a lane (be certain that each sample has been prepared properly with agarose gel loading buffer – see step E above.)
12. Load appropriate molecular weight size markers into a lane.
13. Set the voltage at ~100 V (depending on your particular power unit) and allow the gel to run until dye is 2/3 of the way down the length of the gel.
14. While wearing goggles and a plastic welder's mask place the gel on a UV transilluminator, take appropriate notes and photograph the gel.

Note - Do not be alarmed if you see extra bands in addition to the alleles. DNA heteroduplexes can be expected when performing nondenaturing agarose gel electrophoresis. The sole purpose of the agarose gel is to confirm the success of the PCR amplification of the STR loci.

### Step III: Polyacrylamide Gel Electrophoresis for visualizing the 3 PCR Amplified STR Loci Products using Promega's Silver Stain Detection

Adapted from Promega's *Geneprint*® STR Systems (Silver Stain Detection) – Technical Manual No. D004.

#### Solutions and Materials needed:

glass plates (31.0 cm x 38.5 cm x 0.4 mm)	(user supplied)
sharks-tooth comb and one-well comb	(user supplied)
side spacers, foam pads	(user supplied)
clamps	(user supplied)
electrical tape	(user supplied)
power supply for large PAGE gels	(user supplied)
PAGE apparatus for gels $\geq 30$ cm	(user supplied)
0.5X and 10X TBE buffer	(user supplied)
acrylamide	(user supplied)
BIS acrylamide	(user supplied)
urea	(user supplied)
TEMED	(user supplied)
10% ammonium persulfate	(user supplied)
sterile water	(user supplied)
95% ethanol	(user supplied)
Kimwipes	(user supplied)
bind silane	(supplied with kit)
acetic acid	(user supplied)
Gel Slick	(user supplied)
50-100 cc syringe with hooked needle	(user supplied)
STR 2X loading solution	(supplied with kit)
pGEM DNA marker	(supplied with kit)
CTT allelic ladder	(supplied with kit)
silver nitrate	(supplied with kit)
37% formaldehyde	(supplied with kit)
sodium thiosulfate (10 mg/mL)	(supplied with kit)
sodium carbonate	(supplied with kit)

#### Gel Set-Up, Pouring and Running Procedure:

1. Using Kimwipes clean glass plates thoroughly with 95% ethanol.
2. Mark the two outer sides of the plates (one per plate) that won't be chemically treated.
3. In a chemical fume hood mix together 950  $\mu$ L of high quality ethanol, 42  $\mu$ L sterile water, 3  $\mu$ L bind silane, and 5  $\mu$ L acetic acid.
4. Soak a Kimwipe with the above solution and apply to the side of the long plate not marked in step 2 above. Wait 5 minutes for Bind Silane to dry. Using Kimwipes clean off any excess bind silane with 95% ethanol.
5. Repeat the cleansing step (step 4) 2-3 more times.
6. DO NOT LET THIS PLATE TOUCH THE OTHER PLATE.



7. Add 2 mL of Gel Slick to center of the other plate (the shorter plate). Wipe with a Kimwipe in circular motions to cover the entire plate. Allow the Gel Slick to dry for five minutes. Using a Kimwipe, wipe away the Gel Slick film in circular motions over the whole plate.
8. AGAIN, DO NOT ALLOW THE TWO PLATES TO TOUCH ONE ANOTHER.
9. Position the side spacers (no bottom spacer) and carefully assemble the glass plates together. Place clamps to hold the plates and spacers in place.
10. Using electrophoresis tape, tape the bottom and sides of the plates, making sure no bubbles are present (so as not to allow the acrylamide solution to leak out).
11. Place three clamps along each side of the glass plates but not along bottom.
12. Prepare 4% acrylamide solution by mixing 31.5 grams urea, 40 ml of distilled water, 3.25 ml 10 X TBE, and 7.50 ml of 40 % acrylamide:BIS solution. Filter this solution.
13. Add 50  $\mu$ L TEMED and 500  $\mu$ L 10% ammonium persulfate. Immediately take up the solution in a 60 ml syringe and begin to pour/insert solution between the plates (place plates at an angle).
14. Remove any bubbles using a bubble buster.
15. Insert a one well comb.
16. Place three clamps along top to keep one-well comb in place and allow the plates to sit horizontally for at least 1 hour.
17. Remove clamps, tape and comb once polymerization is complete.
18. Pour 0.5X TBE into bottom chamber of gel tank.
19. Place gel in gel tank (short plate facing the apparatus) and make sure plates are secure with foam pads to prevent leaks.
20. Insert sharks tooth comb approximately 1-2 mm into gel.
21. Pour 0.5X TBE in top chamber of tank.
22. Use needle syringe to remove urea and bubbles in wells and at bottom of plates.
23. Prerun the gel at 1500V, 100mA, and 100W for 30 minutes.
24. While gel is pre-running, prepare samples by adding 2.5  $\mu$ L of the PCR sample to 2.5  $\mu$ L of the STR 2X loading solution. Also, add 2.5  $\mu$ L of the CTT allelic ladder to 2.5  $\mu$ L of the STR 2X loading solution.
25. Heat samples at 95 $^{\circ}$ C for 2 minutes and immediately place on ice.
26. Place power input on standby while loading the gel. Make sure that ladders are placed among the samples for straightforward analysis following electrophoresis.
27. Run gel at 1500V, 100mA, and 100W for about 1.5 hours.
28. Remove gel from gel tank.
29. Carefully pull plates apart (gel should be fixed to the large plate by the action of the bind silane).
30. Place long plate and gel into a tub for the staining procedure.

**Silver Staining Procedure: (Consult the Promega Silver Stain Detection Kit for exact instructions and for solution recipes):**

1. Pour the Fix/Stop solution on the gel and let gel sit for 20 minutes (agitate the solution intermittently). Remove and save the Fix/Stop solution for step 7 below.
2. Pour distilled water on the gel and let sit for 2 minutes. Remove water.
3. Repeat step 2 two more times.

4. Pour staining solution into tub onto gel and let sit for 30 minutes (again, agitate the solution intermittently). Remove staining solution.
5. Pour distilled water into tub and let the gel sit for only 10 seconds. If time exceeds 20 seconds, repeat step 4. Discard water.
6. Add developer solution (4-10°C to gel. Let the gel sit in developer solution for only up to five minutes. Go to step 7 after five minutes or when alleles/ladders become visible on gel.
7. Add fix/stop solution directly to developer solution when alleles/ladders first become visible. Let sit for 5 minutes. Discard solution.
8. Add distilled water for 2 minutes and discard.
9. Position gel upright and let dry overnight.
10. Analyze the DNA fingerprints on the gel.

### C. A description of Hardy-Weinberg population genetics and its statistical significance in DNA fingerprint analysis.

The statistical calculation of an allelic frequency estimate is used in forensic biology to assess the strength of evidence, and the probability that some specific person other than the suspect will leave matching DNA evidence. To determine the likelihood that a specific genotype will occur in a population, many individuals may be tested to determine the probability of that specific genotype occurring, or alternatively the model of Hardy-Weinberg equilibrium may be applied. The Hardy Weinberg equilibrium model explains that gene frequencies, and by extension the genotype ratios, will not change, and mathematically demonstrates how the gene pool frequencies remain stable.

Under this model, observed allele frequencies (instead of genotype frequencies) are used to estimate the genotype at each locus. Several assumptions are made when using the Hardy Weinberg equilibrium model: mutations have not occurred, the number of individuals in the population is large and mating is completely random within a specific closed population group. In reality, there is a tendency for people to mate within their common ethnic groups, so population studies have collected data for several ethnic groups and determined the frequency of specific alleles at the common loci used in forensic biology.

Tables of allele frequencies are available from Promega as well as other sources, for such population types as Caucasian, African-American, and Hispanic, while specific frequencies for certain alleles are published in the Journal of Forensic Science among other print sources. The allele frequencies are used to calculate the genotype frequency for each locus, assuming that the allele is following Hardy-Weinberg equilibrium (*i.e.*, the assumptions described above). The frequency may be calculated for heterozygotic loci as  $2pq$  when  $p$  is defined as the frequency of allele 1, and  $q$  is the frequency of allele 2. When homozygous loci are present, the genotype frequency calculation requires a term called theta ( $\theta$ ), a correction factor needed due to the potential for nonrandom mating in a specific population, where the term  $p^2 + \theta p(1-p)$  calculates the probability, and the value of theta is usually considered to be approximately 0.01 to 0.03 depending upon racial group. Theta is needed because in an inbred population, the error caused by assuming HW equilibrium can overestimate the strength of the evidence and favor the prosecution in magnitude.

In this paper, as well as in the class discussions, HW equilibrium was assumed, and the value of theta was not used as a correction factor. The homozygous frequency is simply defined as  $p^2$ . If time permits in the lectures, the idea of nonrandom mating may be introduced along with the theory behind the statistical calculations. An excellent discussion of the assumptions made and the mathematics may be found at <http://dna-view.com/>, the forensic mathematics web site of Dr. Charles Brenner or in reference 8.

Following determination of the genotype frequency, the frequency of the complete profile is estimated by multiplying the frequency of each locus (product rule). This again assumes no genetic linkage between each locus and random assortment of alleles during mating. The resulting overall profile frequency for even the 3 loci we tested in class was very small, and therefore justifies statements such as “there is a 1/1000 chance that a particular unrelated person would have the same DNA profile”.

## Useful web sites to learn more about Forensics:

1. Promega has an entire division devoted to genetic identity. The kits are used not only in police labs but in paternity testing as well.  
[www.promega.com](http://www.promega.com)
2. Human genome Project- information about CODIS, interesting uses of DNA Forensic identification, ethical issues.  
[http://www.ornl.gov/sci/techresources/Human\\_Genome/elsi/forensics.shtml](http://www.ornl.gov/sci/techresources/Human_Genome/elsi/forensics.shtml)
3. NIST information on STR's, including a powerpoint slideshow that is helpful for lectures  
<http://www.cstl.nist.gov/div831/strbase/>
4. American Academy of Forensic Sciences  
<http://aafs.org/>
5. International Association for Identification  
<http://theiai.org/>
6. An excellent discussion of the assumptions made and the mathematics may be found at <http://dna-view.com/>, the forensic mathematics web site of Dr. Charles Brenner or in reference 8

**Example calculation of allelic frequency:** Here we provide an example allelic frequency calculation for individual number 1 from the gel in Figure 1. Individual number 1 is of African-American ethnicity so the Promega supplied frequency tables (Technical Manual No. D004) for African-Americans was used. For the statistical analysis the formula  $P = 2pq$  was used for heterozygous loci and for homozygous loci the formula  $P = p^2$  was used.

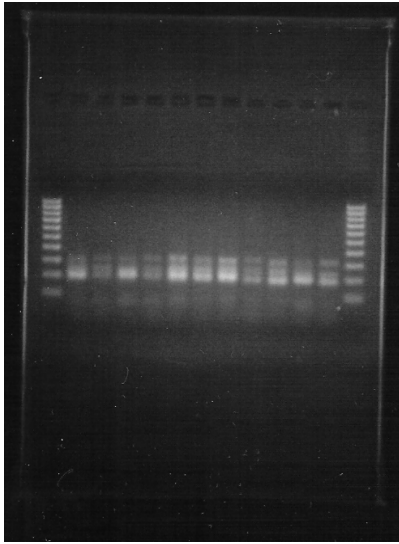
To obtain the allele frequency used for p and q the band numbering for each individual's alleles are first determined by comparing them to the CTT ladder bands on the acrylamide gel (Figures 1 and 2). For example, in Figure 1 individual number 1 had two CSF1PO alleles (bands) at positions 10 and 11. The numbers 10 and 11 correspond to the number of repeats (*i.e.*, STRs) contained in the amplified PCR product. For the CSF1PO loci the smallest number of repeats is 7 and therefore the fastest migrating band (the lowest) in the CTT ladder is assigned the number 7. To obtain the allelic frequency one looks up the population frequency for each band (for a particular ethnic group) in the Promega supplied allelic frequency table. The frequency of the band with 10 repeats in the African-American group is 0.273 and 0.232 for the band with 11. To obtain the probability of an individual possessing both the 10<sup>th</sup> and 11<sup>th</sup> bands one applies the formula  $P = 2pq$  or  $2 * 0.273 * 0.232 = 0.126672$ .

The same calculation is performed for the other two loci. For the TPOX locus individual number 1 had bands at the 9<sup>th</sup> and 10<sup>th</sup> positions which upon looking up in the supplied table of allelic frequencies for African-Americans revealed the frequencies of 0.192 for the 9<sup>th</sup> and 0.113 for the 10<sup>th</sup> band. Using the formula  $P = 2pq$  one obtains the value  $2 * 0.192 * 0.113$  or 0.043392 for the two bands at the TPOX locus. For the TH01 locus individual number 1 had bands at the 8<sup>th</sup> and 9<sup>th</sup> positions which upon looking up in the supplied table of allelic frequencies revealed

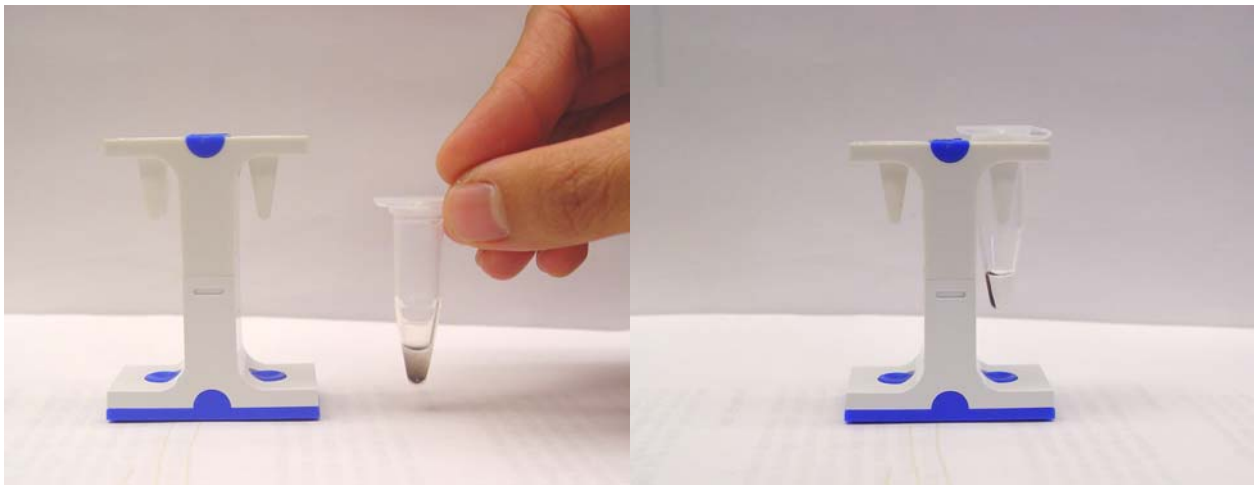
the frequencies of 0.233 for the 8<sup>th</sup> and 0.127 for the 9<sup>th</sup> band. Again, using the formula  $P = 2pq$  one obtains the value  $2 * 0.233 * 0.127$  or 0.059182 for the two bands at the THO1 locus.

Following calculation of probability at each locus, the product rule was applied to calculate the probability of a match across all three loci. For individual number 1 the total probability was calculated by multiplying the probabilities calculated for each set of bands at each loci (*i.e.*,  $P_{CSF1PO} * P_{TPOX} * P_{THO1} = 0.126672 * 0.043392 * 0.059182 = 0.0003253$ ). Therefore, for individual number 1 there is a 1/0.0003253 (or 1 in 3074.11) chance that an individual would randomly match the particular combination of his six alleles of a sample left at a potential crime scene. This calculation was performed for all individuals tested and the results are depicted in Figure 1 and tabulated in Table 1.

**D. Supplemental Figure 1 and Figure 2**



**Supplemental Figure 1:** Confirmation of successful PCR reactions resolved on a 1.5% agarose gel.

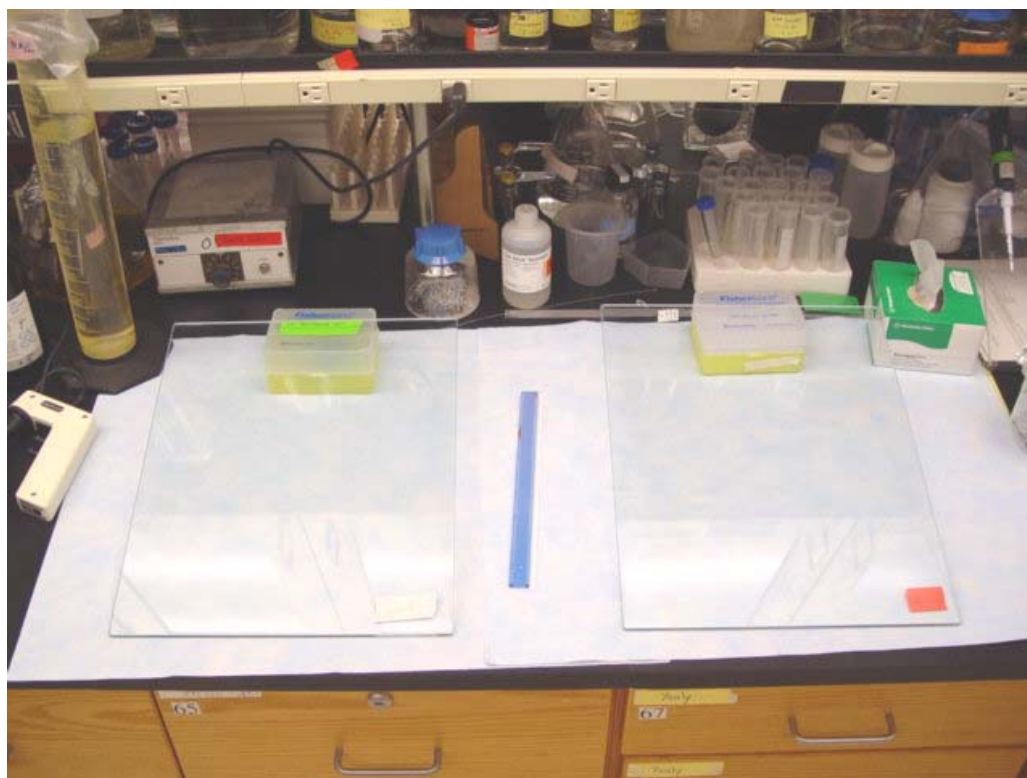


**Supplemental Figure 2:** Genomic DNA isolation using magnetic bead technology as described in Promega's DNA IQ System protocol.

**E. Important Points when Preparing Large Acrylamide Gels and twelve electronic images meant to visually assist with the large polyacrylamide gel setup and pouring.**

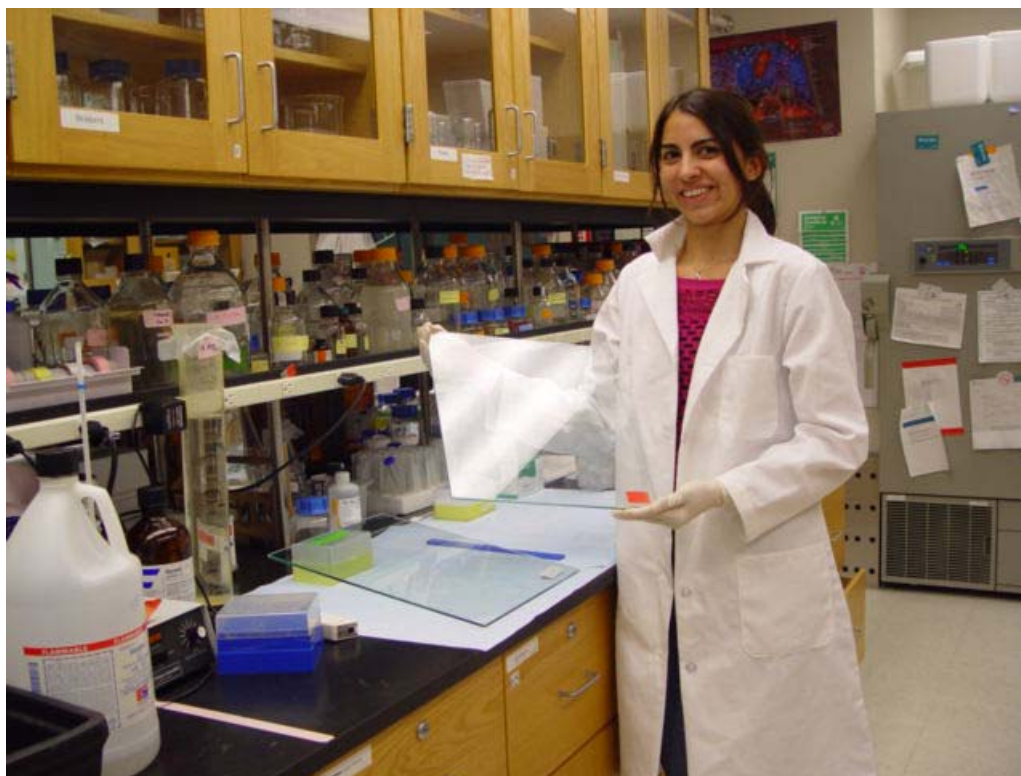
When preparing the large polyacrylamide gel it is imperative to adhere to the following procedures to achieve the desired results. Proper application of the bind silane and the gel slick to the appropriate glass plates is essential to prevent damage to the gel during and after electrophoresis. Trials in which the gel slick was not used or when the treated plates were allowed to come in contact resulted in problems with the gel and failed visualization of the DNA bands. The suggested running time of the acrylamide gel (~1.5 hours) is optimal and results in the positioning of the DNA bands in the center of the gel. The gel running should be terminated when the bromophenol blue dye of the STR 2X loading buffer has just run off the gel and the xylene cyanol green dye should still be visible on the gel. In addition, to obtain maximum staining results for visualization purposes, the students must strictly adhere to the time constraints of the silver staining protocol (especially the five minute limit for applying the developer solution to the gel).

Below are a series of 12 electronic images that were taken during the process of putting the glass plates together, and the subsequent pouring of the large polyacrylamide gel. These images are meant to visually assist those attempting this process for the first time.



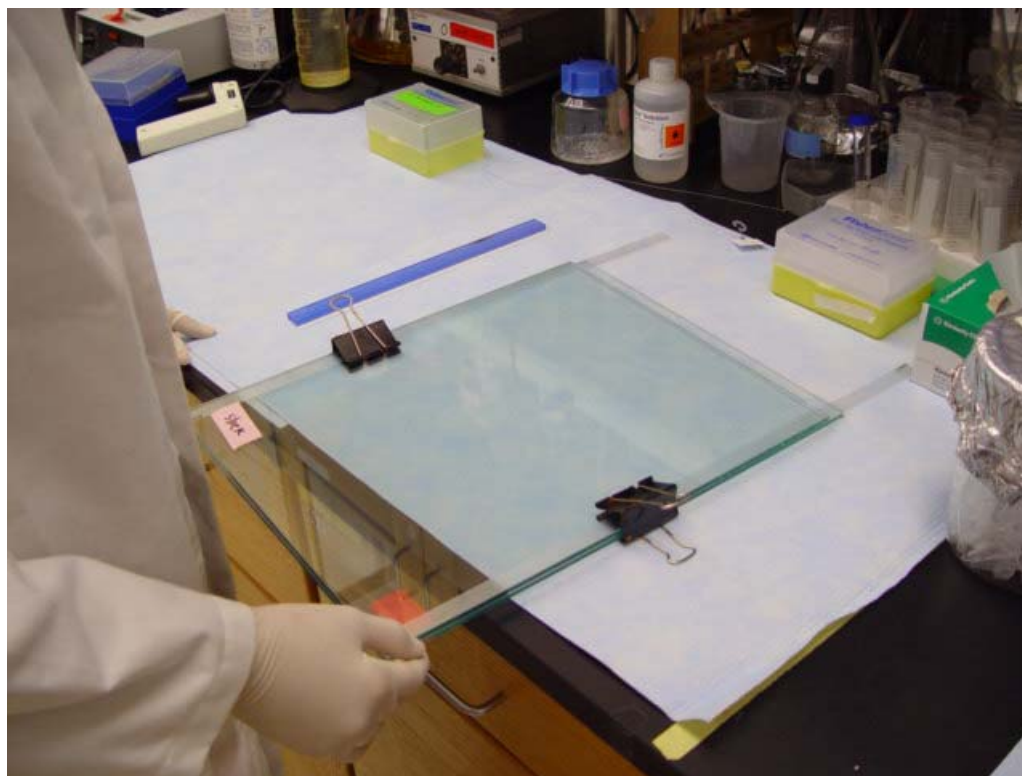
**Supplemental Figure 3:** Begin by taking two large glass plates (31.0 cm x 38.5 cm x 0.4 mm), one slightly smaller than the other and thoroughly wash them with a mild detergent and water followed by a washing with alcohol. After cleansing with alcohol, gently wipe all surfaces with a lint-free tissue, being careful not to touch the surface too much with fingers. This is to ensure the bind-silane and gel-slick, when applied, will uniformly adhere to the glass.





**Supplemental Figure 4:** Make the bind-silane and gel-slick working solutions that are described in the manual and clearly indicate which side of the glass you will not treat. It is also important to mark each glass plate with the solvent used, this will help when pulling the plates apart just prior to staining. Pipette 2-4 ml (depending on plate size) of the bind-silane solution onto one plate and distribute equally over the plate with a lint-free tissue. Repeat this step with the gel-slick on the other glass; this solvent is used to inhibit sticking of electrophoresis gels to glass surfaces. Then cover the plate to prevent dust contamination and leave to air dry on the bench for one hour.





**Supplemental Figure 5:** Once plates are thoroughly dry, lay the bottom plate on the bench, bind-silane face up, and place thin spacers on either side of the glass, as shown in the figure. These thin spacers prevent the plates from coming into physical contact with each other. If the plates touch, it is important to thoroughly wash and re-treat them with their respective solvent. Carefully place the small glass plate on top of the large plate, ensuring that the bottom is perfectly aligned. When properly aligned, securely clamp the plates in place with large clamps.



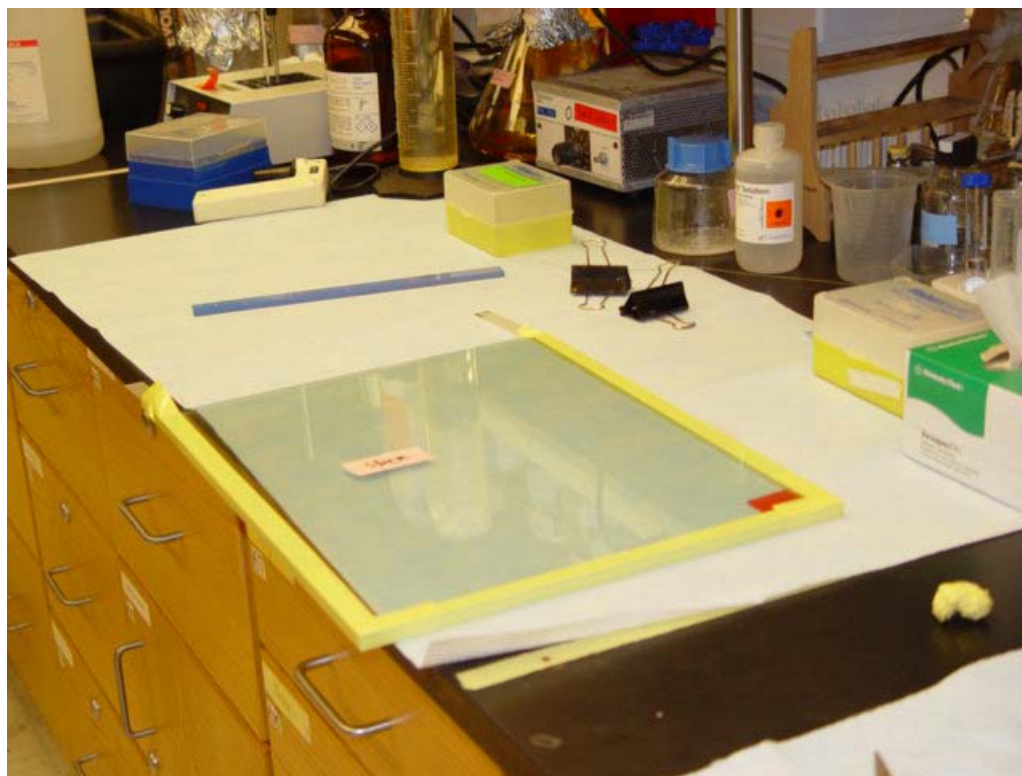
**Supplemental Figure 6:** Carefully remove the clamps from one side and thoroughly tape with highly adhesive electrophoresis tape. It is important to verify that the tape is securely adhered to the glass, otherwise it will leak when the gel is poured between the plates.



**Supplemental Figure 7:** It is recommended to cut the tape the length of the plates and utilize the tape so half will be adhered to the top plate while the other half will be adhered to the bottom plate, as illustrated in above figure. Be sure that while taping, the other sides of the plates remain tightly clamped.



**Supplemental Figure 8:** Again, proper taping on all sides of the glass plates cannot be emphasized enough as liquid acrylamide leakage may occur from improper taping. One should be certain that the tape is tightly adhered on all sides and especially the bottom.



**Supplemental Figure 9:** Once all sides have been taped, the plates should look similar to the taped plates in the above figure. Notice that each glass plate is labeled with different color tape to indicate the respective solvent used.

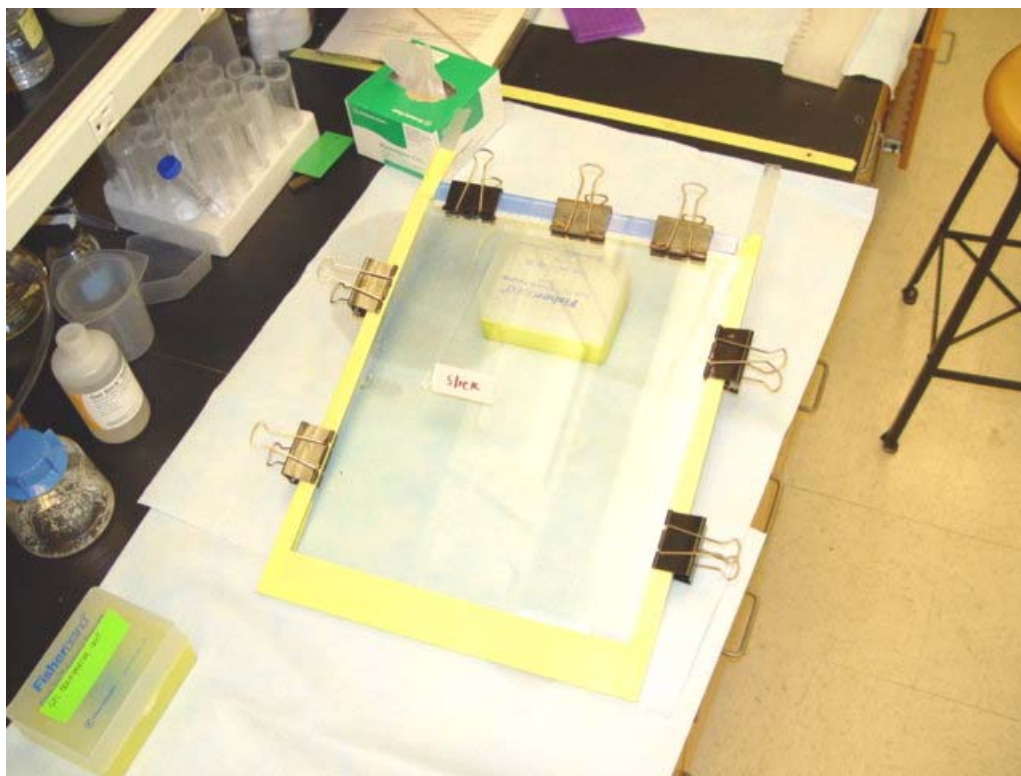




**Supplemental Figure 10:** Once all sides of the plates have been adhered tightly with tape, tilt the plate approximately  $30^\circ$  to the horizontal and slowly pour in the liquid acrylamide between the plates using a 60 mL syringe. It is helpful to begin pouring on one side of the plate while tilting the plates unto one corner until the bottom is filled. This helps prevent bubbles, which will affect how the DNA bands will migrate through the gel.

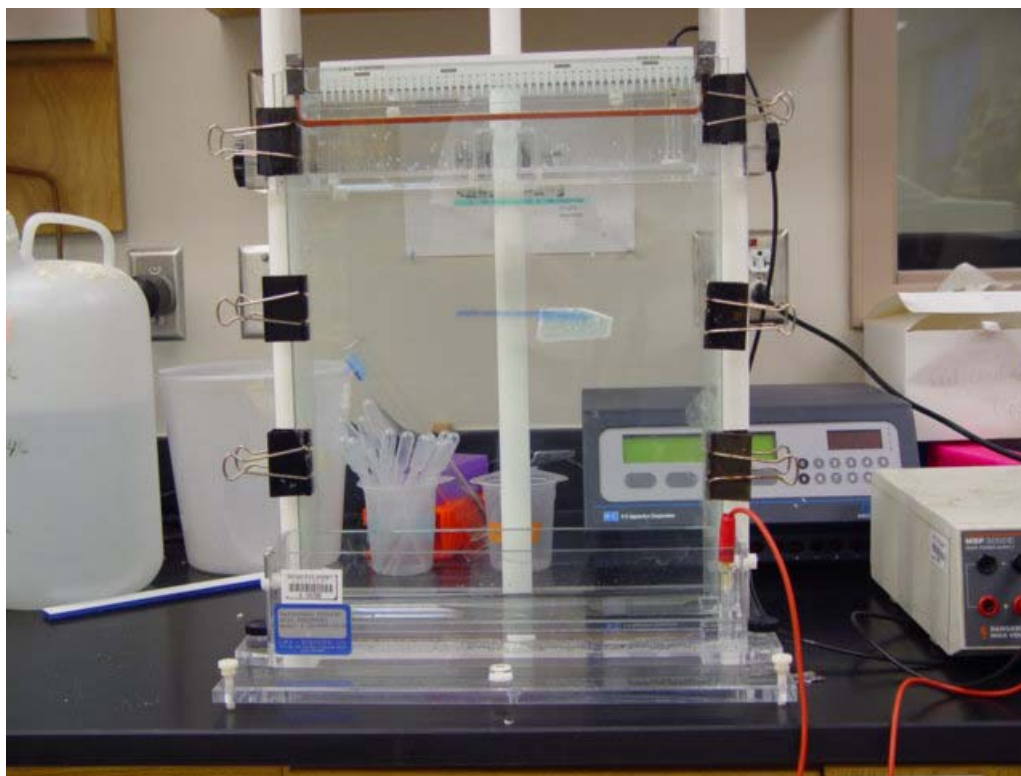


**Supplemental Figure 11:** Once the gel has been properly poured, thoroughly check for bubbles and remove any bubbles with a bubble buster (a thin wire apparatus designed to assist in bubble removal). Then carefully place a one-well comb between the plates at the top.

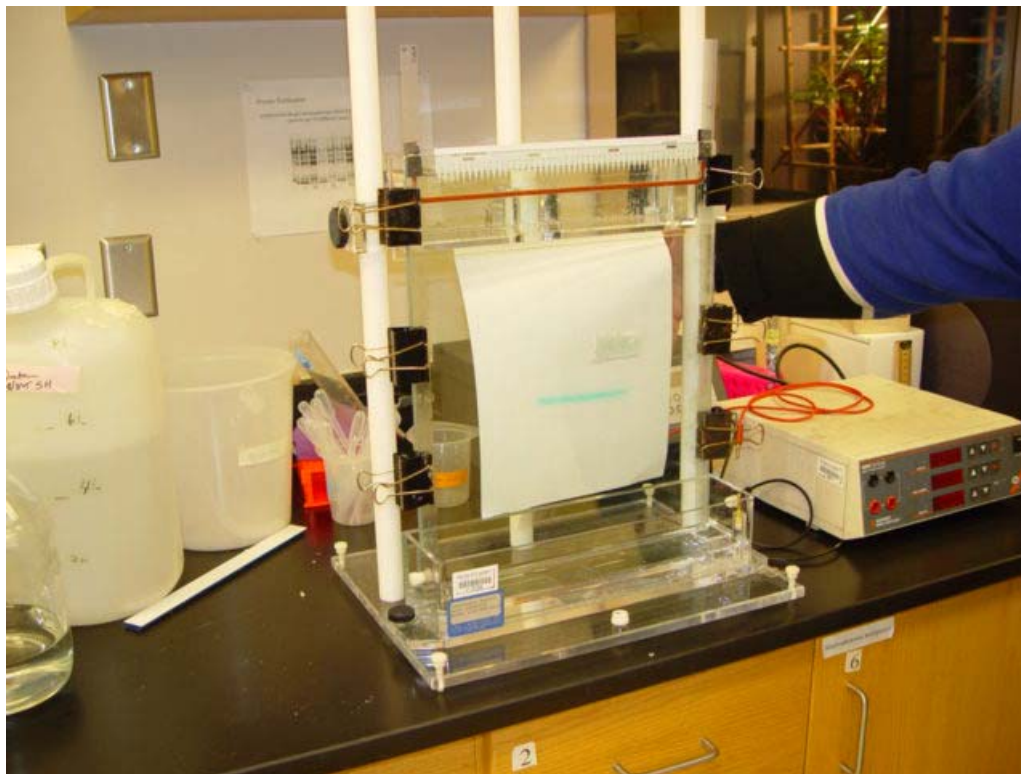


**Supplemental Figure 12:** Once the gel has been successfully poured between the plates and the comb has been placed at the top, the gel is left to polymerize for approximately one to one and one-half hours. Once the gel has polymerized, carefully remove all clamps, tape and the one-well comb.





**Supplemental Figure 13:** At this point there should not be any tape remaining on the plates. The bottom chamber of the gel tank is to be filled with 0.5X TBE and the plates placed in the tank with the short plate facing the apparatus. Be sure to secure plates with foam pads to prevent leaks and insert a sharks-tooth comb. Then proceed to fill the top chamber with 0.5X TBE.



**Supplemental Figure 14:** Be sure to secure the gel into place with clamps on either side and remove excess urea and bubbles with a syringe. Pre-run the gel at 1500V, 100mA, and 100W for thirty minutes while preparing the samples. Finally, load your samples and run the gel at 1500V, 100mA, and 100W for approximately one to one and one-half hours.