Lab 103: DNA Fingerprinting.
STUDENT LABORATORY GUIDE
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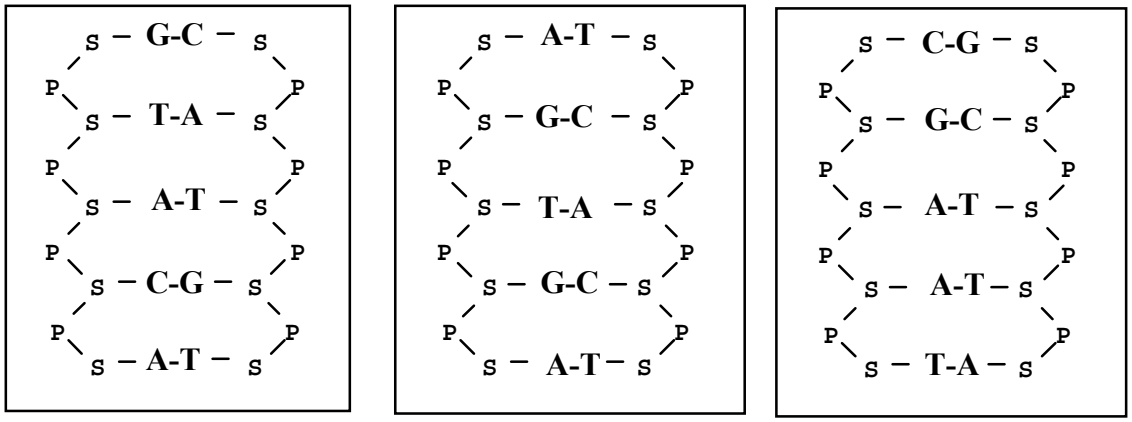
The Task : You are about to perform a procedure known as DNA Fingerprinting. The data obtained may allow you to determine if samples of DNA that you will be provided with are from the same individual or from different individuals. For this experiment, it is necessary to review the structure of DNA molecules.

Structure of DNA



The schematic above represents a very small section of DNA from three different individuals. In this representation of DNA the symbol system is as follows:

Side Chains

S = five carbon **S**UGAR molecule known as deoxyribose

P = **P**HOSPHATE molecule composed of a phosphorous and oxygen atoms

DNA **NUCLEOTIDE** BASES
A = adenine **C** = cytosine **G** = guanine **T** = thymine

Analysis of the three DNA samples above (see next page) might help us detect some of the expected similarities and differences in samples of DNA from different people .

1) Compare the "backbone" of sugar-phosphate arrangement in the side chains of all three figures. Are there any differences?

2) In the above figure, do all three samples contain the same bases? Describe your observations.

3) Are the bases paired in an identical manner in all three samples? Describe the pattern of the base pair bonding.

4) In your attempt to analyze DNA samples from three different individuals, what conclusions can you make about the similarities and differences of the DNA samples?

Upon careful observation, it becomes quite apparent that the ONLY DIFFERENCE BETWEEN THE DNA OF DIFFERENT INDIVIDUALS IS THE ***LINEAR SEQUENCE*** OF THE BASE PAIRS. In lab, your team will be given some DNA samples. Recall that your task is to determine if any of them came from the same individual or if they came from different individuals.

5) What will you need to compare between these DNA samples to determine if they are identical or non-identical?

Developing The Plan: At first sight, your task might seem rather difficult…that is you want to determine if the ***linear*** base pair ***sequence*** in the DNA samples is identical or not! An understanding of some relatively recent **developments in recombinant DNA technology** might help you to develop a plan.

In 1968 a group of enzymes were discovered in bacteria [Dr. Werner Arber at the University of Basel, Switzerland and Dr. Hamilton Smith at the Johns Hopkins University, Baltimore] which when added to **any** DNA will result in the breakage [**hydrolysis**] of the sugar-phosphate bond between certain specific nucleotide bases [**recognition sites**]. This causes the double strand of DNA to break along the recognition site and the DNA molecule becomes fractured into two pieces. These molecular scissors or "cutting" enzymes are **RESTRICTION ENDONUCLEASES.** [Can you figure out why they are called *restriction endonucleases* ?]

Two common restriction endonucleases are BamHI and HindIII [which will be provided to you]. To better understand how BamHI and HindIII may help you in performing your DNA fingerprinting test, first, you must understand and visualize the nature of the "cutting" effect of a restriction endonuclease on DNA :

The line through the base pairs represents the sites where bonds will break if a restriction endonuclease has the recognition site **GGATCC**. The following analysis questions refer to how a piece of DNA would be affected if a restriction endonuclease were to " cut " the DNA molecule in the manner shown above.

1) How many **pieces** of DNA would result from this cut ? _____

2) Write the **base sequence** of the DNA fragments. What differences are there in the two pieces?

3) DNA fragment **size** can be expressed as the number of **base pairs** in the fragment. Indicate the size of the fragments [mention any discrepancy you may detect].

   a) The smaller fragment is _____ base pairs (bp).

   b) What is the length of the longer fragment ? _____

Consider the two samples of DNA shown below [single strands are shown for simplicity]:

Sample #1: **C A G T G A T C T C G G A T C C G C T A G T A A C G T T**

Sample #2: **T C A T G G A T C C C T G G A A T C A G C A A A T G C A**

If both samples are treated with a restriction enzyme [recognition sequence **GGATCC**] then indicate the number of fragments and the size of each fragment from each sample of DNA. Assume that the enzyme cuts in the same way as shown on page 3.

Sample # 1 Sample # 2

# of fragments:_____ # of fragments: _____

List fragment size in ascending order: largest ----> smallest

Sample # 1 Sample # 2

Thus far, your preliminary analysis has included the following:

- The similarities and differences between the DNA from different individuals
- How restriction endonucleases break [hydrolyze] DNA molecules
- How adding the same restriction endonuclease to two samples of DNA might provide some clues about differences in their linear base pair sequence.

Once you have some fairly clear understandings about these three items you are ready to proceed to the first phase of DNA fingerprinting procedure - performing a restriction digest on your DNA samples.

Materials per team:

Crime Scene DNA 5 µl [0.2 µg/µl]	Restriction buffer, [10X]	P-10 micropipet
Suspect 1 DNA 5 µl [0.2 µg/µl]	Rack for reaction tubes	Waste container
Suspect 2 DNA 5 µl [[0.2 µg/µl]	Permanent marker pen	Clear tips
Suspect 3 DNA 5 µl [[0.2 µg/µl]	Power supply	Loading dye
Suspect 4 DNA 5 µl [[0.2 µg/µl]	Weigh boats	Ziploc™ bags
Electrophoresis box [for gel analysis]		

Materials per class:

RE BamHI enzyme [on ice]	TAE buffer	Microcentrifuge
RE HindIII enzymes [on ice]	Incubator at 37°C	Agarose [0.8%] !HOT!
Ethidium Bromide staining solution	Microwave oven	UV trans-illuminator
Polaroid camera, filter & hood	Polaroid 667 film	Latex gloves

CAUTION: KEEP ENZYMES ON ICE!! [Enzymes are inactivated at room temperature!]

Procedure -- Read very carefully !

❑ 1. Obtain the five tubes of DNA (Crime Scene [CS], Suspect 1 [S_1], Suspect 2 [S_2], Suspect 3 [S_3], Suspect 4 [S_4]) Put you name and period number on the tubes! The restriction digests will take place in these tubes. These tubes may now be kept in your rack. These tubes do not need to be on ice. Record your observations of these samples, below:

Observations

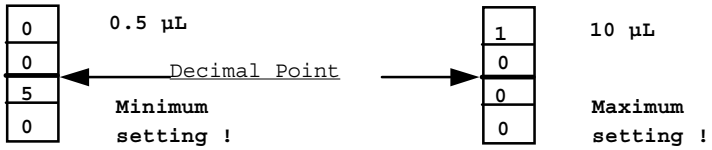
1) Describe the samples of DNA (physical properties)

2) Is there any observable difference in the samples of DNA?

3) Describe the appearance of the restriction endonucleases.

❑ 2. Add reagents to each reaction tubes as shown in the matrix below

DNA SOURCE	RE BUFFER	HindIII ENZYME	BamHI ENZYME
Crime Scene [CS]	7.5 µl	1.0 µl	1.0 µl
Suspect 1 [S_1]	7.5 µl	1.0 µl	1.0 µl
Suspect 2 [S_1]	7.5 µl	1.0 µl	1.0 µl
Suspect 3 [S_1]	7.5 µl	1.0 µl	1.0 µl
Suspect 4 [S_1]	7.5 µl	1.0 µl	1.0 µl



- **Change tips when you change reagents** or if the tip touches the liquid in the tubes **accidentally**. WHEN IN DOUBT, CHANGE TIPS! Buffer goes in the tube before enzyme. ***ALWAYS ADD ENZYME LAST.***

❑ 3. Close the caps on all the tubes. Pulse [2 sec] in the microfuge to mix and combine reactants. (Be sure the tubes are in a **BALANCED** arrangement on the rotor. Use a blank tube, when needed, to balance [provided by your teacher].

❑ 4. The restriction digests will incubate at 37°C for a few hours. The digests will be stored in the refrigerator until the next lab period. Record your observations of these samples, below:

Observations

Describe any visible signs of change in the contents of the tubes containing the DNA combined with the restriction enzymes:

DNA + BamHI :

DNA + HindIII:

Analysis

1) Thus far, can you see any evidence to indicate that your samples of DNA were fragmented or altered in any way by the addition of BamHI or HindIII? Explain .

2) In the absence of visible evidence of change, is it still possible that the DNA samples were fragmented? Explain your reasoning.

3) **After a 24-hour incubation period**, are there any visible clues that the restriction enzymes may have in some way changed the DNA in any of the tubes? Explain your reasoning.

Our task now becomes clear. We must some how get evidence to answer the following question --->**Do the BamHI and HindIII restriction sites occur at the same locations in any of the DNA samples?** Since we are attempting to detect changes at the molecular level, and there are no visible clues for us to analyze, this task might seem beyond our capabilities and impossible to do. Let's see if we can figure this out.

One way to determine the location of restriction sites is to elucidate

1) how many different sizes of DNA fragments are in each sample and
2) what are the relative sizes of each fragment.

Gel Electrophoresis

The following background information might be helpful to you in your attempt to determine the range of DNA fragment sizes in your samples. This technique separates fragments according to their relative size. It may become easier to understand how this technique works if you first cast you agarose gel.

❑ 5. Casting an agarose gel.

A. Raise the ends of the casting tray and tighten its tiny screws. Insert a 6-well comb. Place the casting tray on a paper towel [in case it leaks].
B. Pour 20-25 mL (should be just hot enough to hold) agarose into the casting tray.
C. Let the agarose solidify to a gel [do not disturb the casting tray for 10 min]. While waiting for the gel to form, label a plastic weigh boat with your name and class period.

After your gel has formed, consider the following analysis:

1) Describe the texture of the solid gel.

2) How does the texture of the gel change before and after cooling?

3) The electrophoresis apparatus creates an electrical field [positive and negative ends of the gel]. DNA molecules are negatively charged. To which pole of the electrophoresis field would you expect DNA to migrate ? (+ or -)

4) What color represents the negative pole?

❑ 6. Add 2 µl of loading dye to each tube, as shown in the matrix:

DNA TUBES	loading dye
Crime Scene [CS]	2 µl
Suspect 1 [S_1]	2 µl
Suspect 2 [S_1]	2 µl
Suspect 3 [S_1]	2 µl
Suspect 4 [S_1]	2 µl

❑ 7. When the gel has solidified, lower and secure the "gates" at either end of the casting tray to the down, fixed position.

❑ 8. Place the casting tray on the platform in the gel box. The wells should be at the (-) cathode end of the box, where the **black**-lead is connected.

❑ 9. Pour 300 mL of electrophoresis buffer from carboy.

❑ 10. Pour buffer in the gel box until it ***just covers*** the wells.

❑ 11. **Gently** remove the comb from the solidified gel. Take care that you do not tear the wells. Use either the 6 or 12 well comb depending on your team size and the total number of gels per class.

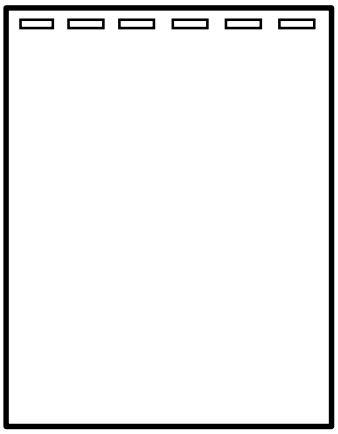
❑ 12. With the 10-µl micropipet, load **10 µl** of each sample in **separate** wells.
Generally, gels are read from left to right. The first sample is loaded in the well at the left-hand corner of the gel.

❑ 13. Secure the lid on gel box. Connect electrical leads power supply. Each channel has two outlets: red for [+] and black for [-].

❑ 14. Turn on the power supply. Set to 100V. Electrophoresis starts.

❑ 15. The process of electrophoresis usually requires 40 to 50 minutes. This can be done in two sessions. The first session should be for at least 10 min. or until the bi-colored loading dyes (purple and blue) have moved out of the wells.

While your DNA samples are moving through the gel, consider the following analysis questions



1) DNA samples were loaded in wells and were "forced" to move through the gel matrix. Which size fragment would you expect to move toward the opposite end of the gel most quickly? Explain.

2) Which fragments are expected to travel the shortest distance [remain closest to the well]? Explain.

3) Mark three bands on the diagram [above] to indicate where you expect the following DNA fragments to migrate: 1000 bp, 2000 bp, 4000 bp. Mark the bands in the third lane and label them according to size.

❑ 16. When it is time to stop, turn off the power and disconnect the leads from the power supply.

❑ 17. To save the gel for viewing at another time, lift the casting tray out of the gel box and CAREFULLY slide your gel to its labeled weigh boat.

❑ 18. Mark a Ziploc™ bag with your name. Slip the weigh boat containing your gel into the Ziploc™ bag. Refrigerate till next class period.

Completing The Electrophoresis

❑ 19. Carefully return the gel to a casting tray on which the "gates" have been lowered and secured.

❑ 20. Place the tray into a gel box. Add buffer to the proper level (see # 11).

❑ 21. Slide the lid on the gel box. Connect the electrical leads to the power supply.

❑ 22. Turn on the power supply. Set it for 100V [current: 80 milliamps]

❑ 23. Continue the electrophoresis until the total time of 40-50 minutes has been reached or long enough to achieve adequate separation of the blue and purple marker dye fronts.

While electrophoresis is in progress, proceed to familiarize yourself with photography of the gel. In the final two steps, you will: [A] ***visualize*** DNA fragments in your gel and [B] ***analyze*** how the number and positions of visible DNA bands on ***your*** gel relate to the ***central question*** **: Are any of the DNA samples from the same individual?**

Making the DNA visible

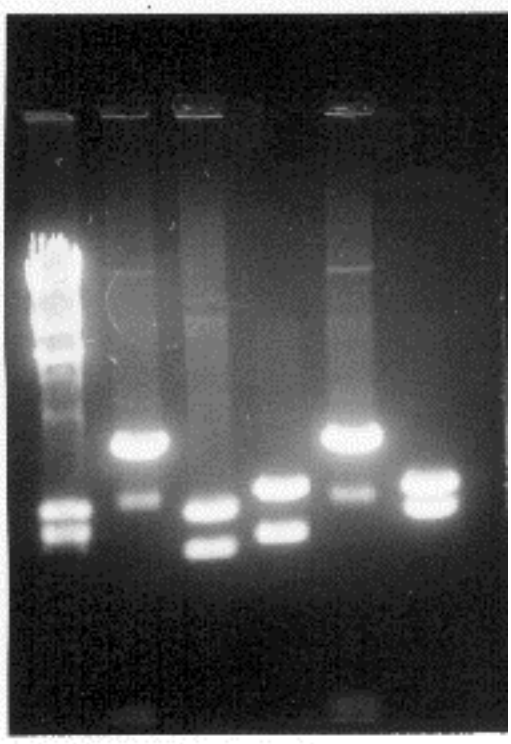
- Unaided visual examination of gels indicates only the positions of the loading dyes and <u>not</u> the positions the DNA fragments. Check to see if this is also true with your gel. **<u>Describe what you see.</u>**

- DNA is visualized by staining the gel in Ethidium Bromide [EtBr] solution. EtBr **intercalates** between DNA base pairs [like food particles between your teeth after a meal]
- Ultraviolet radiation excites the electrons in EtBr which results in visible fluorescence. EtBr complexed with DNA in the gel matrix, therefore, appears as visible bands upon exposure to UV source. In this **indirect** manner, DNA is made visible.
- These visible bands of DNA-EtBr may be photographed for analysis.

<u>CAUTION</u>: Wear gloves. Prevent EtBr or gel stained with EtBr from coming in contact with any part of your body! Wear gloves.

The photograph at the right shows a gel stained with EtBr. For fingerprinting analysis, the following information is important:

- Each lane has a different sample of DNA
- Each DNA sample was treated with the same restriction endonuclease(s)



With reference to the numbered lanes, start to analyze the bands in the photograph in the section below.

Refer to pages 3 & 7, if you have forgotten about the relationship of restriction sites, number, size and relative position of DNA fragments in a gel.

Lane --> 1 2 3 4 5 6

Analyzing the bands

1. What can you assume is contained within each band?

2. If this were a fingerprinting gel, then how many kinds (samples) of DNA [can we assume] was placed in each separate well? How many did you place in each well.

3. What would be a logical explanation as to why there is more than one band of DNA for each of the samples

4. What caused the DNA to become fragmented?

5. Which of the DNA samples have the same number of restriction sites for the restriction endonuclease used? Write the lane numbers.

6. Which sample has the smallest DNA fragment?

7. How many restriction sites were there in sample three?

8. Which DNA samples appear to have been "cut" into the same number and size of fragments?

9. Based on your analysis of the photograph, what is your conclusion about the DNA samples in the photograph? Do any of the samples seem to be from the same source. If so which ones?_____ Describe the evidence that supports your conclusion.

Saving the Gels for Photography and Analysis

❑ 24. Turn off the power supply. Disconnect the leads.

❑ 25. Lift the casting tray out of the gel box

❑ 26. CAREFULLY slide the gel on the labeled weigh boat. Seal with the same Ziploc™ as before.

❑ 27. Decide where to store the gel.

Staining with Ethidium Bromide -
Students should not do the "staining" step!
Ethidium bromide is a proven carcinogen and mutagen.

❑ 28. Bring the weigh boat containing the gel (from the previous lab) to the staining station. Have your gel stained with a dilute solution of EtBr. This procedure takes about 10 minutes. The gels must then be rinsed in distilled water---you teacher will do this!

Viewing and documenting gels - your teacher will provide the directions for this process

❑ 29. Have your teacher transfer your gel to the surface of the UV trans-illuminator.

Students should not do this step! EtBr is a proven carcinogen and mutagen. CONSULT YOUR TEACHER!.

When the lid is closed, the trans-illuminator radiates UV light through the gel. DNA-EtBr adducts will emit visible fluorescence.

❑ 30. Observe your gel on the UV trans-illuminator. **Only one photograph per gel will be taken! Your teacher will make Xerox copies of the photograph for other members of the team.**

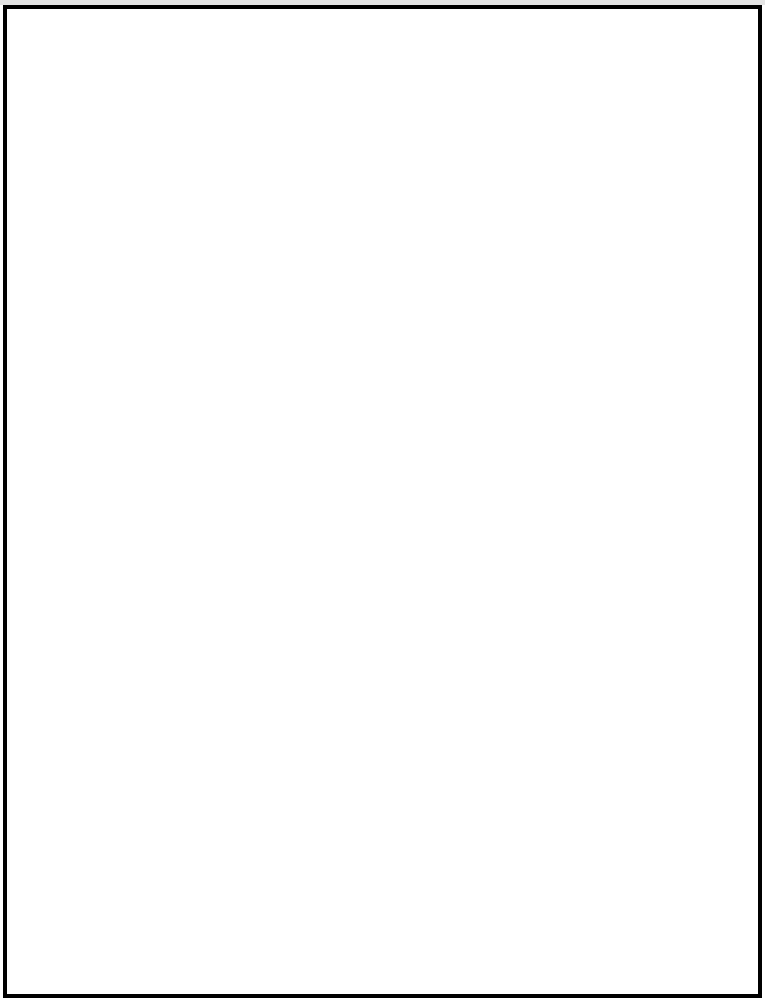
❑ 31. A Polaroid photograph of the gel should be made at this time.

❑ 32. Upon completion of photography, dispose gels, staining solution and weigh boats in the prescribed manner.

ANALYZING YOUR DATA

Attach a photo or Xerox of your gel. This is your experimental data.

Indicate which sample is in each well. ---------->



1) What are we trying to determine? Re-state the **central** question.

2) Fill in the chart below. It will facilitate data analysis.

DNA Source	Number of DNA Fragments	Distance Migrated by Each Fragment (mm)	Number of Restriction Sites

3) Which of your DNA samples were fragmented? What would your gel look like if the DNA were not fragmented?

4) What caused the DNA to become fragmented?

5) What determines where a restriction endonuclease will "cut" a DNA molecule?

6) A restriction endonuclease "cuts" two DNA molecules at the same location. What can you assume is identical about the molecules at **that location**?

7) Do any of your suspect samples appear to have BamHI or HindIII recognition sites at the same location as the DNA from the crime scene?

Conclusion

Based on the above analysis, do any of the suspect samples of DNA seem to be from the same individual as the DNA from the crime scene? Describe the scientific evidence that supports your conclusion.

TEACHER'S LABORATORY MATERIALS GUIDE

1. Formulas for buffers can be found in *DNA Science*, Micklos and Fryer, Carolina Biological Supply Company.

2. Plasmids, enzymes and diluent can be obtained from various supply companies; New England Bio., Wards, Carolina, Promega, etc.

3. This kit uses Bam HI and Hind III. Look at restriction maps for other plasmids for cutting sites using different enzymes...for example, you may want some plasmid to give three fragments when cut.

4. Try to purchase Ethidium Bromide in solution. The powder is very fine and there is danger of inhaling it! If you have a hood and wish to prepare your own solution use gloves and approved mask. You will need to add 100µL of 5mg/mL Ethidium Bromide solution to 500 mL of dionized or distilled H_2O. Plan location for staining station. Get adult help to stain gels. Under no circumstances should student handle bottles of EtBr. You might investigate using a Methylene Blue stain.....check with BioRad Corp. (Hercules, CA) 1-800-4BIORAD....Some formulations from other suppliers require almost a 10-fold concentration of DNA!

5. Locate a source of ice, bulk ice chest and individual ice buckets (Styrofoam cups).

6. Materials per team:

Crime Scene DNA, 5 µl [0.1 µg/µL]	restriction buffer, [2 X]	p-10 micropipet
Suspect 1 DNA, 5-µl [0.1 µg/µL]	rack for reaction tubes	waste container
Suspect 2 DNA, 5 µl [[0.1 µg/µL]	permanent marker pen	sterile, clear tips
Suspect 3 DNA, 5 µl [0.1 µg/µL]	power supply	loading dye
Suspect 4 DNA, 5 µl [0.1 µg/µL]	weigh boats	Ziploc™ bags
gel box	Ice + "bucket"	

Materials per class:

CAUTION KEEP ENZYMES ON ICE. The enzymes are most stable when kept ice cold.

B*am* HI enzyme, ice cold	TAE buffer	microcentrifuge
H*in d* III enzymes, ice cold	incubator, 37°C	agarose, [0.8%], hot
Ethidium bromide staining solution	microwave oven	UV transilluminator
Polaroid camera, filter & hood	Polaroid 667 film	latex gloves

Sample	Kind of DNA	Amount	Concentration
Crime Scene DNA,	plasmids --varies	2 µl	[0.2 µg/µL]
Suspect 1 DNA,	pUC18, pUC19	2 µl	[0.2 µg/µL]
Suspect 2 DNA,	pUC2.3, pAMP	2 µl	[0.2 µg/µL]
Suspect 3 DNA,		2 µl	[0.2 µg/µL]
Suspect 4 DNA,		2 µl	[0.2 µg/µL]

[1] Russ Janigian (SFUSD), Len Poli (SFUSD), Cindy Murphy-Erdosh (Teacher Education in Biology/SFSU), Shouman Datta (UCSF)