Experiment 2 - Identity Testing

OVERVIEW.

Experiment 2 consists of three laboratories that focus on identity testing. In the first two laboratories you will analyze a crime scene and use DNA evidence to identify a criminal. In the third laboratory you will use the mitochondrial DNA isolated in Experiment 1 to continue to explore and discuss the uses and limitations of using DNA for identity testing. The three laboratories will cover:

Sept 24th/Oct 1st:

First half of class:

Collect evidence from crime scene. Extract DNA from evidence for PCR based analysis. Amplify (by PCR) the DNA from crime scene and suspects.

Second half of class:

Analyze mitochondrial DNA (from experiment 1) by agarose gel electrophoresis.

Oct 8th/Oct 15th:

First half of class:

Analyze crime scene DNA by agarose gel electrophoresis.

Second half of class:

Whole lab discussion and presentation of results and analysis used to solve forensic case. Everyone will write a summary report of their analysis (due October 23rd/30th).

Oct 22nd/Oct 29th: Computer analysis of mitochondrial DNA (from Experiment 1) and discussion of conclusions.

LABORATORY 2.1: CRIME SCENE ANALYSIS

Following up on the news story that just appeared in the local papers (web posting, also available as a handout), you arrive at the crime scene prepared to do an analysis of the evidence. As you walk in, someone has just handed you one additional pieces of paper containing statements from the key suspects (handout). With this information in hand, you set about your task as a forensic scientist specializing in DNA analysis.

Experiment

Reagents

20 µl DNA collected from crime scene
20 µl PCR mastermix, which includes two D1S80 primers, *Taq* polymerase, dNTPs, and PCR buffer
10 µl $MgCl_2$

Equipment

0.2 µl PCR tube
Micropipette and tips

Shared Equipment

Vortex
Thermocycler

Procedure for each group

1. Read article regarding the crime.
2. Draw out the crime scene in your lab notebook.
3. Choose two (2) pieces of evidence for DNA analysis. Mark and label carefully. Use evidence collection and receipt form.
4. Extract DNA from evidence using the same protocol as lab 1.1B
   Can obtain DNA from evidence either by:
   i. Removing a small portion of the evidence and soak it in a tube filled with saline (as you did with the swab from lab 1). Can use sterile razor for this purpose – 70% EtOH is available for sterilization.
   ii. Using a swab moistened with saline to wipe area of evidence where you think DNA is located.

   Use same method for a negative control, i.e. cut off a piece where you think no DNA will be, or wipe the swab in an area where you think no DNA will be, and continue that sample through the same protocol of lab 1.1B.
5. From GSI, obtain: PCR ingredients, and isolated DNA for a positive control.
6. Label your tube-tray with your group name.
7. **Keep all tubes and reagents on ice throughout setup**.
8. Select DNA sample from one (1) suspect.
9. Track forensic analysis through evidence collection form.
10. Set up separate PCR reaction for each DNA sample as follows:
    Label all small PCR tubes with contents, and your group signature.
    Add 20 µl of PCR mastermix to each tube.
    Add 20 µl of DNA sample to each tube.
    Repeat for each DNA sample and controls.
11. Once all samples are complete, add 10 µl of $MgCl_2$ to each sample.
12. Remove all tubes from ice, put in labeled tube-tray, and give to GSI.
13. Give labeled PCR tubes to GSI, to amplify in thermocycler.

Evidence at the crime scene

1. Opened Department of Chemistry envelope addressed to Professor Nick Steneck. Note written in multi-color block letters on a yellow lined sheet of paper, which reads: "DNA = **D**eath is **N**ear, **A**dios. Your days in charge are over, Nick!"

2. Five (5) sealed and crumpled up envelopes addressed to Professor Nick Steneck scattered on the floor.

3. Four (4) cigarette butts.

4. Six (6) empty beer bottles

5. One wrapper from a Wendy's hamburger.

6. Eight (8) Wendy's napkins stained with ketchup.

7. A legal-size partially used writing tablet with yellow sheets of paper.

8. A folder marked with the initials E.B.G.

9. Five (5) chewed on "Sharpie" marker pens, (2 black, 1 green, 1 red, 1 blue).

10. Three (3) half-eaten french fries and part of a ketchup stained hamburger bun.

Post Laboratory Questions (Answer in your lab notebook)

1) Describe the kinds of evidence that might be found at a crime scene from which DNA evidence might be gathered.

2) Discuss what is meant by "evidence chain of custody" and why this is important in DNA forensic evaluations.

3) What are some variables that may cause degradation of a DNA sample and make analysis difficult?

4) Describe how DNA contamination of evidence may take place and discuss what kinds of problems might be associated with DNA contamination of evidence.

5) When doing PCR analysis of DNA evidences, what kinds of quality controls are important and why?

6) What is the difference between a negative control and a positive control? Give an example of each for a PCR based analysis.

7) What considerations must be addressed when collecting DNA specimens from suspects?

Background

- ✓ What is a polymorphism?
  - It's a variation or alteration in DNA sequence that has no negative impact on an individual.
  - Only a small percentage of our DNA is polymorphic
  - Most polymorphisms used in identity testing are not located in genes but in less conserved regions of the genome.
  - Provide us with a unique DNA fingerprint.
  - May be a single base change, an addition of DNA, or changes in DNA tandem repeat copy numbers.

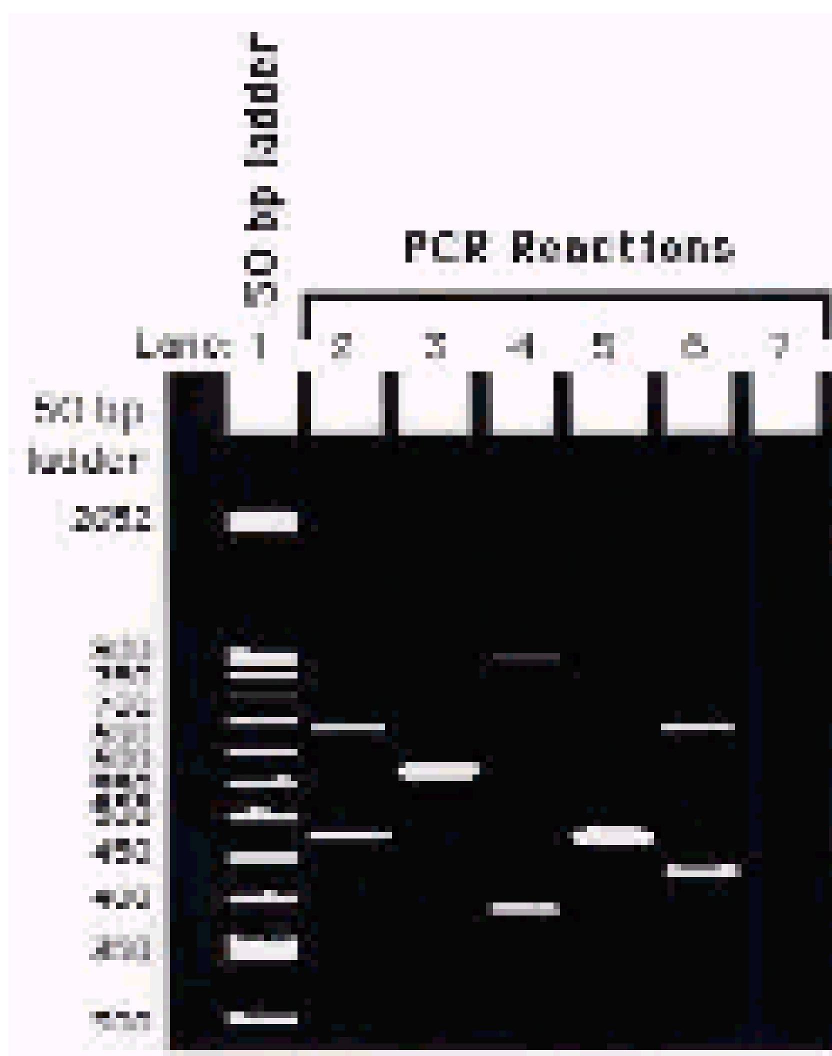
- ✓ What is a VNTR?
  - Variable Number Tandem Repeat
  - Length polymorphism
  - Repeat unit is usually between 5 and 64 base pairs

    ATCGAATCGA**ATCGA**ATCGA**ATCGA**

  - Function, if any, remains unknown
  - Number of repeated units varies from chromosome to chromosome
  - VNTRs with large number of alleles in the population, the chance of any two unrelated individuals carrying the same two alleles is very low, often less than 1%.
  - The more independent loci that we look at, the chance that individuals share the same alleles at all of the loci becomes extremely small.

- ✓ What are the properties of D1S80
  - VNTR
  - Located on chromosome 1p
  - Size of repeat unit is 16 bases
  - Repeats range in size from 350 bp to 1000 bp
  - >27 identified alleles in the world
  - Range of repeats varies from 15 to 41
  - 77% of Caucasions are heterozygotes
  - Power of discrimation = 94%-96%
  - FBI uses 13 STR loci to have extremely high power of discrimation.

  www.dps.state.mn.us/bca/lab/documents/dnabroc2.pdf


50 bp ladder
PCR Reactions
Lane: 1 2 3 4 5 6 7

Laboratory 2.2A: PCR Analysis by Gel electrophoresis

Experiment

Reagents

2.5% agarose gel
D1S80 allelic ladder
Loading buffer
Amplified DNA samples

Equipment

1-20 µl pipettes and tips

Shared Items

Electrophoresis chamber
Power supply

Procedure

❖ *Ethidium bromide is a mutagen! Please wear gloves and avoid contact with skin while loading the gel.*

1. Mix 2µl of loading dye with 8 µl of PCR sample.
2. Load 10µl of sample into well of gel.
3. Electrophorese at 120 volts for 60 minutes.
4. Visualize gel under UV light, and document results.

Post laboratory Questions (Answer in your lab notebook)

1. Describe what a DNA polymorphism is and how identification of polymorphisms are useful in DNA identity and forensic testing.

2. Describe three (3) different classes of DNA polymorphisms that may be used in DNA testing.

3. Describe three (3) different methods that may be used to detect DNA polymorphisms in forensic or identity testing.

4. Describe ways in which PCR conditions can affect your results.

5. Describe ways in which specificity of your PCR reaction can be improved.

6. Describe ways in which the yield of your PCR reaction may be improved.

LABORATORY 2.2B: SOLVING THE CRIME: WHODUNNIT???

Experiment

1. Determine which two alleles are present in your DNA samples.
2. Using this information, as well as your classmates' results, determine the guilty party through class discussion.

Post-laboratory Questions (Answer in your laboratory notebook)

1. What criteria will you use to determine a match between the perpetrator of the crime and the suspects being evaluated?

2. Are you able to identify who the perpetrator of the crime is by your analysis alone? Why or why not?

3. Is examination of other laboratory data generated by other students in the laboratory helpful in determining who the suspects of the crime are?

4. If this were a real case, what would you do to more conclusively prove your results?

5. How should samples of suspects be handled once they are excluded as being the perpetrator of the crime?

6. How would the CODIS data bank be useful to you if you had access to it if this were a real crime?

7. Discuss both the positive and negative applications and implications of maintaining a national forensic database. Who should be included in such a database? Why?

8. Do your results show any evidence of DNA contamination? Why or why not?

Guidelines for Case Report Assignment

(DUE: OCTOBER 22rd/29th, 25% of lab grade)

Each person will write a case report discussing the crime scene analysis in detail. The case report should be a **minimum** of 3 double-spaced typewritten pages and a **maximum** of 5 double-spaced typewritten pages. Margins should be 1" on each side (I will measure!) and use 12 pt Times New Roman font.

Purpose:

1. To demonstrate understanding of DNA identity testing and polymorphisms.
2. To gain scientific writing skills.

Sections to Include:

1. Introduction – Information regarding DNA identity testing, specifically DNA fingerprinting as it pertains to the crime scene. Include information on the use of DNA polymorphisms, specifically VNTRs and the D1S80 locus.
2. Objective – One paragraph describing the goal of the laboratory exercise.
3. Basis for Evidence Collection - The scientific reasons that you chose your particular pieces of evidence.
4. Materials and Methods – **In paragraph form**, describe the materials used and the procedures used including PCR and gel electrophoresis.
5. Results – Should **only** be the results obtained in lab. **DO NOT INCLUDE CONCLUSIONS OR ANALYSIS IN THIS SECTION!**
6. Discussion – analysis of your results and discussion of your findings, including the reasoning for determining the guilty suspect. If you don't have results, you should hypothesize why this occurred. Apply your findings to the real world, i.e. power of exclusion.

Grading Criteria:

Organization =1/4 of grade
This includes the overall layout of your paper and that information is in the appropriate subheading.

Content = 5/8 of grade
Include all **pertinent** information.

Grammar and Spelling = 1/8 of grade
Spell-check!

LABORATORY 2.3: GENETIC ORIGINS EXERCISE

Experiment

What does your Mitochondrial DNA tell you?

The sequences of the mitochondrial DNA are back and it is now your turn to analyze them.

Looking at your sequences.
For this lab, you will need to open the link to your DNA sequence as well as 4 other students in the lab. To access your sequences, use the tool "Sequence Server" at http://www.bioserver.org/sequences/.

After logging in to Sequence Server, you should click the "Manage Groups" button at the top of the screen. This will open the Manage Groups window. In this window, choose "Classes" from the popup menu on the upper-right. A new screen will appear, and you will be able to see your class's name in the list presented (Angie Lanie). To select your class, click on the checkbox next to "Angie Lanie", and click the OK button. This will put your class on your worksheet.

To compare sequences, you need to have more than one sequence on the worksheet. To add a sequence, select the desired sequence from the popup menu at the bottom of your class. Do this for as many sequences as you want (open your sequence and at least 4 others from the class). Then, click the checkbox for each sequence you wish to include in a comparison.

Open your sequence and look at the quality of the data. When the sequence cannot be determined, the computer uses an "N".

Questions (Answer in your laboratory notebook)

1. Do you have good sequence information across the length?

2. What do you think are some of the variables that affect the quality of the sequence data?

3. Do you think that a poor sequence can be used in forensic studies? Why or why not?

CLUSTAL alignment.
Next you are going to align your sequence with at least 4 of your classmates to get a feeling for how close they are. To do this, you will perform a CLUSTAL alignment of your sequence plus at least 4 adjacent sequences. Make sure that the desired sequences are checked (left). Toggle to CLUSTAL W and push "Compare."

This program will analyze the first 500 bases of sequence (default parameters). In this version of the program, when the sequences are identical, they will not be shadowed. Sequences that vary within a position are in yellow. When the sequence cannot be determined, the computer uses an "N". Sequence positions where there is an N are shadowed in grey.

Questions (Answer in your laboratory notebook)

4. Is your mitochondrial sequence identical to anyone else in your dataset? Are their two identical sequences in your datasets? If they differ, by how many nucleotides are they different? (Use that information and compare to the phylogenic tree that you will draw soon).

5. Are the differences between different students at the same nucleotide position, at different ones, or both?

Phylogenetic tree

Sometimes it is more helpful to visualize the sequences based on their relatedness. You can do this with your set of sequences (you plus 4 more students) by toggling to "phylogenetic tree." To which student is your mitochondrial DNA most similar to? (*try both the cladogram and the phenogram, and* ***draw them in your laboratory notebook***).

BLAST search

Submit your DNA sequence into a database of all mitochondrial sequences (BLAST). Select your sequence (left) and run BLAST.

Now do a BLAST search on the other 4 sequences.

Questions (Answer in your laboratory notebook)

6. To which sequence are you most related?

7. Are all 4 student's sequences equally similar to the same database sequence? Would you expect them to be?