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Does Race Exist? *Alu* Transposable Elements and Ancestry DNA

Peggy Brickman[1] and Jim Burnette[2]

[1] Plant Biology Department, University of Georgia, Athens Georgia 30602 USA
[2] College of Natural and Agricultural Sciences, University of California Riverside, Riverside CA 92507 USA
(brickman@uga.edu; james.burnette@ucr.edu)

Alu transposable elements are useful as DNA markers for human population studies. For example, one *Alu* insertion exists in human populations as 4 different polymorphisms. Interesting variations in the presence of the different alleles in sampled populations from Africa, South America, Europe, and Asia can be used to elucidate human ancestry. In this lab, students examine the genetic basis of race and consider how much genetic variability exists between human populations. Students also experience the excitement of extracting and analyzing their own DNA, performing PCR, restriction enzyme digestion, and gel electrophoresis. The lab can be accomplished in 2 2-hour sessions.

Keywords: *Alu*, transposable elements, ancestry

Introduction

DNA technology is one of the more difficult topics to teach via inquiry: proper technique is critical for success but challenging to master, so implicit instruction is a must. This DNA exercise is very teacher-centered in the aspect that the teacher provides students with a specific question to be investigated, as well as the procedure and desired layout for communicating their results. However, students perform the inquiry activities of determining what will constitute evidence, examining resources in order to make predictions and interpret their results, so it is also very student-centered.

The objectives of this investigation are to:

1. Develop a thorough understanding of how DNA can be extracted and amplified from a human being, and how that DNA can be analyzed by restriction enzyme digestion and gel electrophoresis.
2. Explain how these tests can provide evidence of ancestry, and predict the outcome of their tests based on previously published studies.
3. Collect data from an experiment and analyze that data to refute their prediction.
4. Communicate what the results of the experiment indicate about their ancestry and how valuable this type of evidence is for examining human genetic variation.

Time Table

Week 1:

- Pre-lab, students read a *Scientific American* article about the genetics of race and answer questions.
- In-class, student groups choose a position on the debate over gathering racial data for medical studies and write examples of evidence that supports their position.
- In-class, students extract their DNA from cheek cells and set up a PCR reaction.

Week 2:

- Pre-lab, students use data from the (Kass, et al., 2007) paper to describe the restriction fragment polymorphisms for the *Alu* element they have amplified.
- In-class, students perform restriction enzyme digestion of their PCR products.
- In-class students perform gel electrophoresis, photograph their gels, and begin to analyze results for a summary essay.

Student Outline

Pre-Lab Homework (Week 1)

Read the article: Does Race Exist? by Michael J. Bamshad and Stephen E. Olson, *Scientific American*, December 2003 and answer the following questions before lab.

1. **Context**:
   a. Imagine you are at a party and someone pipes up, "But of course, <insert ethnic group> are < choose characteristic, smarter than, more athletic, more thrifty, more hard-working> than <insert other ethnic group>; it's in their genes." Using what you read in the article, how would you explain what is known about the utility of social definition of race, such as skin color or other physical features, and the underlying DNA evidence of how closely related two human beings are.

   b. According to the authors, why would it be useful to define race from a biological standpoint?

   Give one example used in the article where knowledge of racial differences has been used to benefit humans.

   c. In the article, what are the reasons given to continue using self-reported ancestry as a diagnostic tool for physicians rather than using genetic testing?

   d. What is the function of *Alus* in the human genome, and what characteristic of transposons makes them perfect tracers for genealogy?

   e. Of the total genetic variability within the human species, approximately what percentage of variation can be found within a population, say in Europe? How much variation exists between populations, say Europeans as compared to Asians?

2. **Method**: In your own words, define a polymorphism. Use two made-up DNA sequences to help support your definition.

   Explain how researchers could analyze polymorphisms such as *Alus* to determine ancestry.

3. **Results**: Give two examples of how researchers have documented polymorphisms that result in effects on health.

In-Lab Writing Assignment (Week 1):

The authors of the pre-lab reading make the point that there are racial differences in both susceptibility to diseases as well as in the effects of some therapies. A recent debate has ignited over an FDA (Food and Drug Administration) recommendation that researchers collect survey information about a test patient's race and ethnic identity. Some argue that outward signs of race are not adequate for distinguishing a person's genetic characteristics. Others suggest that we need to know this information to help understand how genetic and environmental differences among groups contribute to disease. During class today, your group will choose a position on the debate, and each person in the group will explain, in his/her own words, examples of evidence that supports your group's position.

Although you should discuss this as a group, your written work should be your own and will be graded as such by your instructor. Be sure to address the following four issues and respond to each with a 2-3 sentence response:

Issues:

- How important are genetic differences in susceptibility to disease?
- What research findings support racial differences in susceptibility to disease or in response to medication?
- How could historical abuses associated with categorizing people by race effect your argument?
- What is the problem with using common definitions of race based on skin color when trying to infer a person's genetic heritage?

Experimental Design

Carry out your experiment and take notes below describing what you did and how your experiments helped you achieve your objectives. Your descriptions should be clear and complete enough so that someone else can follow your logic and repeat your tests if necessary. **Although you should discuss this as a group, your written work should be your own and will be graded as such by your GLA.**

When you are done, please place used swabs in the containers provided. You may dump your tubes into the trashcan.

Objective: What are your experiments designed to find?

Prediction: What sort of results do you expect to see?

Procedure: Instructions for extracting DNA are provided on the next page. Briefly indicate the purpose of each step so that you remember what you did for your final reports. Rinse, Swab, Spin, NaOH base, Heat, Add Tris:

In-Lab Procedure (Week 1): Extracting Human Genomic DNA and PCR Reaction:

1. Rinse your mouth out well with water from the fountain in the hallway.

2. Use a sharpie pen to label two large microcentrifuge tubes (1.5 ml) and a smaller 0.2 ml tube with the your initials on the top cap as well as on the side of each tube. Mark one 1.5 ml tube with: "DNA" and the other with "1/10 dilution". Take the smaller 0.2 ml tube and place a white numbered dot on the top, write your name on the sheet next to your number, and then give it to your instructor.

3. Observe your instructor's lesson in using pipettors. Once you have mastered the technique, move to the next step.

4. Pipette 500 µl of water into the "DNA" tube using the **blue-topped pipettor** and a **blue tip**. Discard the tip in the container provided.

5. Using the swabs your GLA gives you, vigorously rub 20 times on each inner cheek.

6. Put swab in your "DNA" tube with the 500 µl of water. Twirl the swab vigorously. Remove swab and discard in beaker of bleach. Snap the tube shut.

7. Flick tube with finger. The solution should be cloudy.

8. Place "DNA" tube in centrifuge with a partner's tube on the opposite side to balance. When all student tubes are in the centrifuge, your instructor will set the machine to spin for 2 min at 14,000 RPM. When you pull out your tube, you will see a 'pellet' of cells at the bottom and the liquid 'supernatant' on top.

9. Pour the liquid supernatant from your tube into the waste bucket provided, being careful not to dislodge the pellet at the bottom of the tube.

10. Using the yellow pipettor and the smaller tips, add 20 µl of DNA Dilution buffer (DIL tube in ice bucket at your bench) to the pellet in your "DNA" tube. Carefully replace the cap tightly.

11. Tap the tube to dislodge the pellet from the side.

12. Place your tube for 5 min on the bench in your rack.

13. While you are waiting for your "DNA" tube to be heated, take your second tube (the one that has your initials and says 1/10 dilution) and using the **yellow pipettor** and **yellow tips**, place 90 µl of water into it. Discard the tip into the trash.

14. Place your "DNA" tube into the 95°C heating block for 5 minutes.

15. Carefully remove your "DNA" tube and spin again for 2 minutes.

16. Pipette 10 µl from your "DNA" tube and add it to 90 µl of water in the "1/10 dilution" tube.

17. Your instructor has placed 49 µl of PCR reaction mix into your 0.2 ml tube with the white dot. Retrieve this tube and add 1 µl of your "1/10 dilution" DNA to the tube and place in the PCR machine.

Pre-Lab Homework (Week 2)

You learned in last week's lab that *Alu* transposable elements are useful as DNA markers for human population studies. In fact, there are over 5000-7000 new *Alu* insertions into the human genome since our ancestors split from a common ancestor with chimpanzees. Therefore, some humans may not have a specific insertion. In other cases, some of the new insertions may have accumulated mutations that result in polymorphisms that can be used as markers to identify ancestry. Researchers David H. Kass, Nicole Jamison, Melanie M. Mayberry, and Eillen Tecle (Kass, et al., 2007) identified a single *Alu* element that exists in human populations as 3 different polymorphisms. These polymorphisms are changes in the DNA sequence that must have occurred accidentally in different human populations since the time they migrated to different global regions. They sampled populations from Africa, South America, Europe, and Asia for these polymorphisms and uncovered variations in the presence of the different alleles that could be used to elucidate ancestry. The PCR primers that you used last week amplified those *Alu* transposons. This week your restriction enzyme digestion of these *Alu* elements will identify which you inherited.

1. **Context**:

The nucleotide sequence of the *Alu* element that you amplified last week in lab can be found in human populations with one of the following four polymorphisms (S1, S2, and L, YB8 is the ancestral copy):

```
     62     70 73
YB8 _GGCCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGTGGATCATGAGGTCA
S1  T.....GGCGCC.............................................................
S2  T.......T..A.............................................................
L   T.......T..A.............................................................

                                          172
YB8 GGAGATCGAGACCATCCTGGCTAACAAGGTGAAACCCCGTCTCTACTAAAAATACAAAAAATTAGCCGGGCGC
S1  ....................................A.....................................
S2  .........................................................................
L   .........................................................................

            217                 236-237       250    257
YB8 GGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGCGTGAACCCGGGAAGCGG
S1  ............................TA............G......G...................
S2  ............................TA............G......G...................
L   ..........A..................TA............G......G...................

                       298                                       338
YB8 AGCTTGCAGTGAGCCGAGATTGCGCCACTGCAGTCCGCAGTCCGGCCTGGGCGACAGAGCGAGACTCCGTCTC
S1  ...................C.....................................................
S2  ...................C.....................................A.............
L   ...................C.....................................A.............
```

a. Assuming that these polymorphisms occurred following random genetic changes (mutations to the original ancestral sequence (YB8). How many changes occurred in each?

Table 1. Nucleotide Changes in Each Polymorphism

Polymorphism	# Nucleotide Changes
YB8 » S1	
YB8 » S2	
YB8 » L	

b. The changes in the *Alu* element above can be thought of with a plagiarism analogy: Imagine a professor gets 4 nearly identical responses to an in-class writing assignment, and he wishes to figure out what evidence in the writing may uncover the story of what occurred. One student, John, submits a nearly perfect paper. The second student, James, submitted a nearly identical paper to John's but he has a missed word, number 300, that renders the text meaningless at that point. The third student, Julie, submitted a paper identical to James' with the same mistake at word 300, but she also has a new mistake at word 1100. Finally, the fourth student, Susan, has the same identical error at word 300, but she has a new mistake at word 124. In this scenario, the professor could try to determine the order of the plagiarism. Obviously, the papers were all copied in some way, and one would have to assume that the introduction of errors occurred during the copying. So, the professor can start to chart out (as shown below) what might have occurred before he confronts the students:

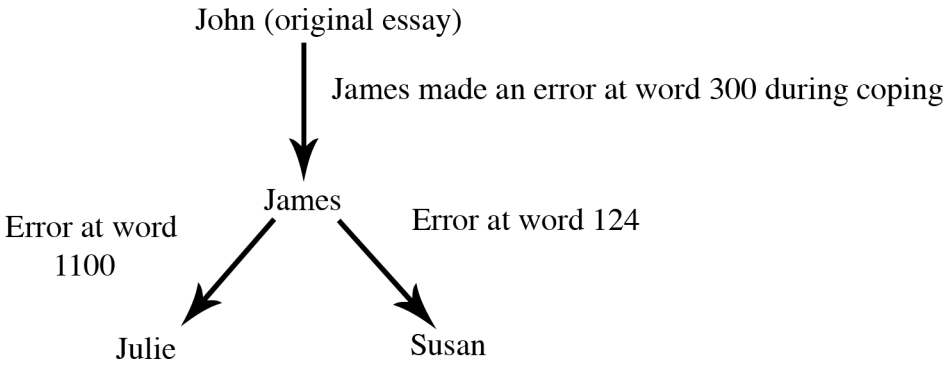

Figure 1: Plagiarism Analogy. The professor could match the changes in each essay that then see if this pattern was supported by their seating arrangements.

This is exactly how scientists compare DNA sequence changes in these polymorphisms. Random errors must have occurred in the copying mechanism during DNA replication, and the descendents of those humans would have inherited the errors. One can then use the errors like the professor did to chart the most logical series of steps. Using the YB8, S1, S2, and L sequences construct a map like the one above that charts the changes in the evolution of the original YB8 element in human populations. Be sure to mark your chart with each of the four elements and describe each mutational change.

2. **Method**:

The nucleotide changes in the four different polymorphisms for this *Alu* allele can be distinguished by DNA sequencing. A fast but expensive way to determine which two alleles you have is to send your PCR reactions off to be sequenced. However, a cheaper and just as effective way to determine which polymorphism you have uses two other techniques, restriction enzyme digestion and gel electrophoresis. These can be done right here in your laboratory. Remember that restriction enzymes are able to cut DNA into pieces at very specific sequences. For example, the restriction enzyme KasI recognizes the sequence GGCGCC underlined in the sequence from homework question #1. Using the sequence from question #1, fill in the following table to indicate how many times the enzyme would cut each polymorphism allele S1, S2, and L, and how many pieces would be generated.

Table 2. Number of enzyme cleavage points and size of restriction fragments generated

Polymorphism	# Times Enzyme Would Cut	# Pieces Made
S1		
S2		
L		

3. **Results**:

Using the following figures of the no *Alu*, S1, S2, and L *Alu* polymorphisms that were amplified by PCR last week, mark the location of the KasI sites with a vertical line and indicate the nucleotide number, so you can visualize your answers from above.

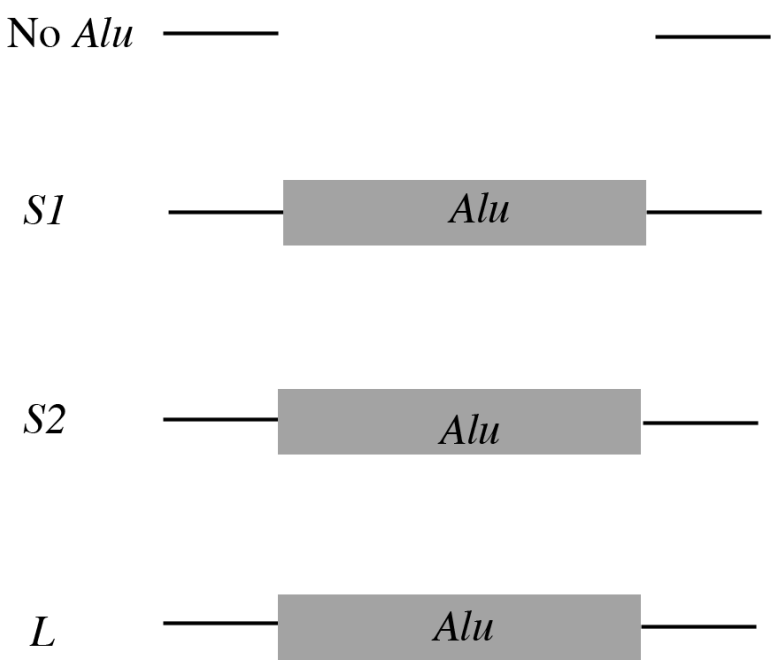

Figure 2: Location of KasI sites in the Four *Alu* Polymorphisms

In-Lab Procedure (Week 2):

Restriction Enzyme Digestion of amplified DNA sample and gel electrophoresis

During the last lab, you extracted your own DNA and performed PCR on your YB8 *Alu* elements. Today you will do a restriction enzyme digestion of your YB8 *Alu* elements. You will use gel electrophoresis technique to separate your restriction fragment pieces by size and then visualize them using a DNA dye. Your objectives for today are to understand your results and to prepare to write an explanatory document for your family.

1. Get a 1.5 ml microcentrifuge tube and place green dot on the top with your same number from l last week. Give your tube to your instructor. They will place 4.7 µl of restriction enzyme mix into your tube. Then, take your green-dot tube from this week plus your white-dot tube from last week back to your bench.

2. Take 30 µl of your PCR reaction and add it to your green-dot tube with your initials and "Digest" on it.

3. Flick tube with finger to mix the contents. Shake once to get the contents back into the bottom of the tube, and place your green-dot "Digest" tube to the 37°C heating block.

4. After 5 minutes, remove your tube from the 37°C heating block.

5. Add 5 µl of 6X Loading Dye to your green-dot "Digest" tube.

6. Add 5 µl of 6X Loading Dye to the remainder of the PCR sample in the white-dot tube.

7. Give both tubes to your instructor to load onto the gel in the order of the numbers you were given. Your instructor will load molecular weight markers in the first lane.

8. *Gel Electrophoresis* (*electro* as in electricity + *phoresy*, movement) is the movement of charged molecules in an electric field. In this case, your DNA fragments are large molecules that will be loaded into small wells cut into a sponge-like agarose gel. An electric current will be applied, and the negatively-charged DNA will move through the spongy matrix of the gel to get to the positive charge on the opposite end of the gel. Large molecules move slower than small ones so that molecules of different sizes appear as separate bands; the smaller DNA fragments move farther through the gel toward the opposite positive end.

9. Run gel 30 minutes at 160 V.

10. Photograph gel under UV light. Several different dyes can be used to bind to the DNA in the gel so that the gel can be visualized after it is photographed. We will be using gel red. Be sure to follow the instructions on the photographic set-up. UV light is a carcinogen, so care must be used to avoid exposure.

In-Lab Writing Assignments (Week 2)

Experimental Design Part 1

While you carry out your restriction digest with help from your instructor, your objective will be to determine a prediction about what your results will show. You will make this prediction based on Table 1 from the Kass et al. 2007 paper (below).

Objective: What are your experiments designed to find?

Table 3. Frequency of Polymorphisms in Different Populations

Group	**-*Alu***	**+*Alu* S1**	**+*Alu* S2**	**+*Alu* L**
African Americans	17.6%	41.2%	23.5%	17.6%
Asian	73.7%	10.5%	0	15.8%
European Caucasian	55.9%	0	26.5%	17.6%
South American	52.9%	0	14.7%	32.4%
Indo-Pakistani	50.0%	11.1%	0	38.9%
Kenyan	15.4%	65.4%	11.5%	7.7%
Chinese	58.3%	16.7%	0	25.0%
Druze	100%	0	0	0
Nigerian	20.0%	40.0%	20.0%	20.0%
Melanesian	75%	25%	0	0

Prediction: You can see from Table 3 that certain polymorphisms are more predominant depending on what part of the world the population was sampled. Using what you know about your family's ancestry, what prediction would you make about which polymorphism you probably inherited. (If you don't know your family's ancestry, choose one group, and describe which polymorphisms would be most likely and least likely to be found in the sample.)

Can you reconcile what you mapped out as an answer to pre-lab question 1b with the data from Table 1?

Every human is diploid, so you have two homologous copies of every chromosome in your body. Thus, you have two copies of the *Alu* allele. Remember the four *Alu* alleles are as follows: - (no *Alu* allele), S1, S2, or L.

List all the possible genotypes that could exist in a population:

Go back to your answers to question 3 from the pre-lab, and for each allele (S1, S2, and L), indicate the sizes of the DNA fragments that would be generated from restriction enzyme digestion of each allele on Figure 3 below.

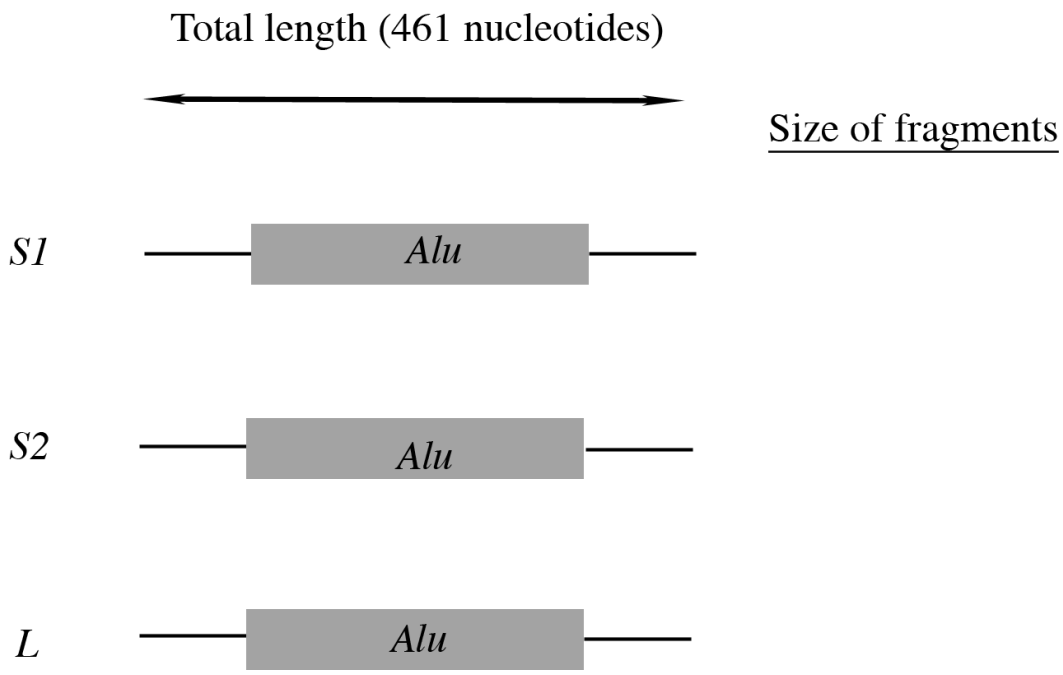

Figure 3. Restriction Fragments Generated from the 3 alleles. Using these lengths and considering a person with the L allele on one chromosome and the S1 allele on the other chromosome (L/S1 genotype), indicate the number and size of all fragments created by KasI digestion of their PCR sample.

After loading your samples onto an agarose gel but while your gel is running, you will use the results of your predictions from above to fill out the drawing of a gel on the next page with the different sized fragments you would expect to see for different genotypes. The drawing should help you interpret the results of the gel electrophoresis.

Experimental Design Part 2:

After completing the first part of this experimental design, you should have:

1. Used your knowledge of your ancestry and Table 1 on page 10 to make a prediction about whether or not you would have the YB8 *Alu* allele, and if you did, which of the 3 polymorphisms (S1, S2, or L) you would most likely have.
2. Created a list of all the possible genotype combinations of these 4 alleles (-, S1, S2, and L).
3. Determined the sizes of the restriction fragment pieces that would be generated for each allele (S1, S2, and L).

Figure 4:

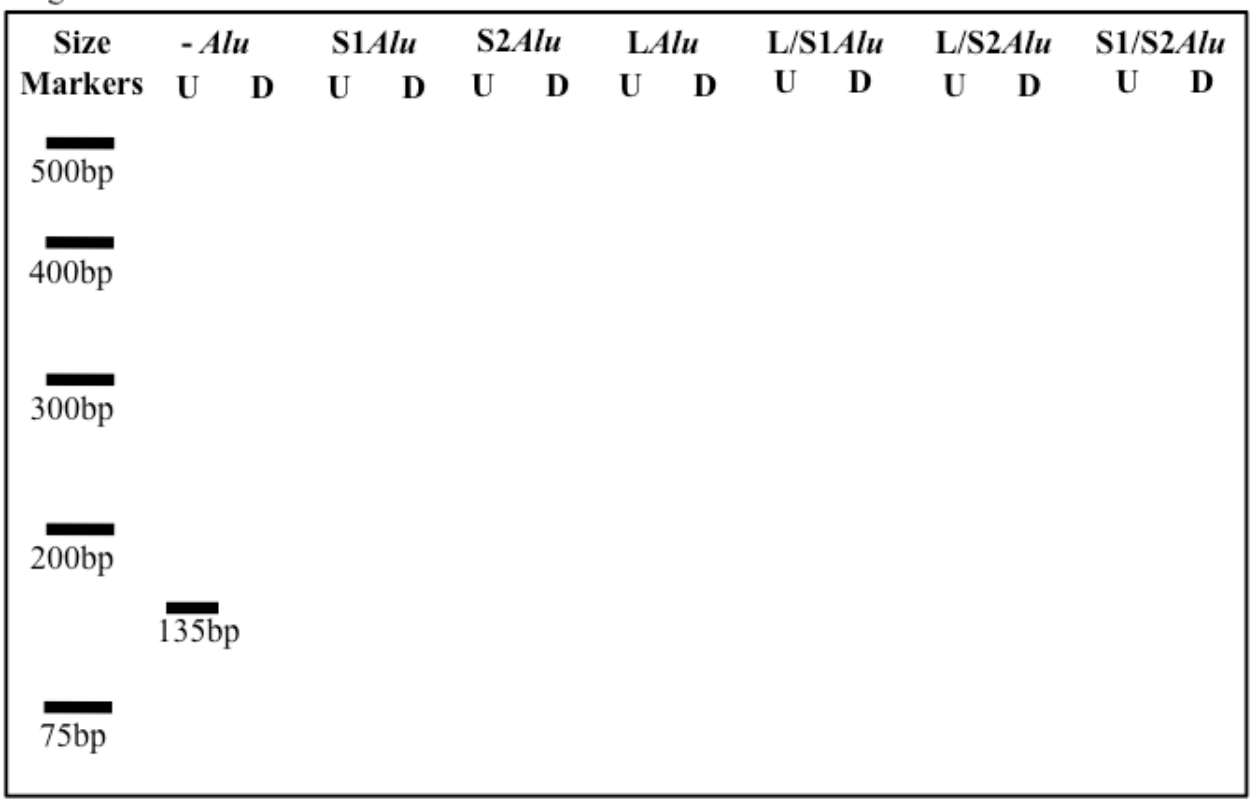

Figure 4. Gel Electrophoresis of the PCR fragments generated. Using just the ends of the sequence without the *Alu* element is 135 bp and is shown in the second lane. Fill in the expected sizes for the restriction digests of the remaining *Alu* elements. (U indicates undigested, and D, digested.)

Experimental Analysis

Evidence: Describe the results of your experiments. How many bands did you find and which pattern do they match?

Explanation: Explain why you think you saw these results. Are they consistent with your prediction based on the evidence from Table 1 on ancestry?

Writing Assignment for the FIRST Draft of the Does Race Exist Summary Essay

Assignment

This is the first draft version of your **Does Race Exist** summary essay (worth up to 10 points). When commenting on this draft, your GLA will focus on large-scale issues related to your summary. This feedback and a subsequent peer discussion will allow you to revise your draft into a well-written final version.

Format of essay

- Your paper should be approximately 3-5 pages in length, including figures and tables. Each figure and table should not take up more than half a page in length. **It does not need to follow a standard format for lab reports; you may write it as you would a general essay.**
- Your draft summary paper must be **typed and double-spaced, font size 12 and font style Times New Roman. Hand-written assignments will not be accepted or graded.**
- **Keep in mind this paper will be longer than 2 pages.**
- **Imagine you are writing to your family to inform them about what your results indicate about your ancestry. Your paper should contain the components listed below.**

Rubric

- Include a title page with your name, lab section (lab – time, day), instructor name, names of group mates, and the academic term. **Up to 0.5 point**

- Important background information keeping in mind that this paper should be interesting and relevant to a non-scientist audience. Provide background information to your family member so that s/he understands why your study is important. For instance, what information about transposons and polymorphisms does your family need in order to understand and be interested in your experiment? Aside from ancestry, what is the benefit to mankind of defining race? You will need to balance scientific terminology with text that does not read like a list of glossary-defined definitions. **Up to 2 points**

- Introduce your prediction about which polymorphism you probably inherited by revising what you wrote for the pre-lab assignments about the underlying DNA evidence of how closely related two human beings are. How useful is the YB8 *Alu* element for predicting ancestry? What was your prediction for your genotype? Explain your reasoning. **Up to 2 points**

- How did the techniques you used (DNA extraction, PCR, gel electrophoresis) allow you compare *Alu* polymorphisms? **Up to 1 points**

- What were the results of your experiment? A photograph of your gel must be included and should have clear labels. Explain how you can infer your genotype from the gel. How did your genotype compared to predicted results? **Up to 2 points**

- Describe what you can conclude from the data you collected. Was your prediction supported? How much confidence can you put in this method? Where does the data indicate your family is from? **Up to 2 points**

- Essay follows correct conventions for grammar and spelling. Ideas are organized in a logical format. Paper is clear and concise. **Up to 0.5 points**

Rubric for FINAL draft of Does Race Exist summary essay

Assignment

You have designed and completed an experiment that determined your genotype for the YB8 *Alu* transposable element. Now write an informative, scientific essay of your journey through this lab. You can earn up to 30 points on this assignment. Remember, if you successfully completed a rough draft and received feedback from your instructor, be sure to hand that draft in along with your final version. **Failure to resubmit this graded rough draft will result in a 10-point deduction from your final grade on the summary. Also, if you do resubmit the draft but fail to make any revisions, a 10-point deduction from your final grade on the summary will be given.**

Format of Essay

- Your paper should be approximately 3-5 pages in length, including figures and tables. Each figure and table should not take up more than half a page in length. It does not need to follow a standard format for lab reports; you may write it as you would a general essay.
- Your final summary paper must be typed and double-spaced, font size 12 and font style Times New Roman. Hand-written assignments will not be accepted or graded.
- Keep in mind this paper will be longer than 2 pages.
- Imagine you are writing to your family to inform them about what your results indicate about your ancestry. Your paper should contain the components listed below.

Rubric

- Include a title page with your name, lab section (lab – time, day), instructor name, names of group mates, and the academic term. **Up to 1 point**
-
- Keeping in mind that the reader of this essay is a family member without scientific background, provide background information to introduce why your essay is important enough to write about. For instance, what information about transposons and polymorphisms does your family need in order to understand and be interested in your experiment? Aside from ancestry, what is the benefit to mankind of defining race? What other information is necessary for your family to understand the importance of your study? You will need to balance the introduction of scientific terminology with text that does not read like a list of glossary-defined definitions. **Up to 4 points**

- Introduce your prediction about which polymorphism you probably inherited by revising what you wrote for the pre-lab assignments about the underlying DNA evidence of how closely related two human beings are. How useful is the YB8 *Alu* element for predicting ancestry? What was your prediction for your genotype? Explain your reasoning. **Up to 6 points**

- How did the techniques you used (DNA extraction, PCR, gel electrophoresis) allow you compare *Alu* polymorphisms? **Up to 4 points**

- What were the results of your experiment? A photograph of your gel must be included and should have clear labels. Explain how you can infer your genotype from the gel. How did your genotype compared to predicted results? **Up to 6 points**

- Describe what you can conclude from the data you collected. Was your prediction supported? How much confidence can you put in this method? Where does the data indicate your family is from? **Up to 6 points**

- Essay follows correct conventions for grammar and spelling. Ideas are organized in a logical format. Paper is clear and concise. **Up to 3 points**

Materials

Per class of 25 students, with 5 groups of 5 students

Chemicals

Item	Concentration	Amt. or # of tubes
Sodium Hydroxide (NaOH)	50 mM (2 g NaOH in 1 L distilled water)	5 tubes with 1 ml each
Tris, pH 8.0	1M	5 tubes with 500 µl each
Sterile water		5 tubes with 600 µl
(PHIRE from NEB: 200 reactions for $280) (Inquire about education pricing.)		
Primers: *Alu*Yb8NBC225F 5'GAGTCCAGCCCATTTTAGCATGGGACA-3' *Alu*Yb8NBC225R 5'-CCCAGCACAAACATGTCATT-3'		
BanI (Cheaper substitute for KasI) with 10X buffers	FastDigest from Fermentas (FD1004) also available from NEB Catalog # R0118S or R0118L	
Loading Dye	6X: 10 mM Tris-HCl (pH 7.6) 0.15% orange G (Sigma:O3756) 0.03% xylene cyanol FF (Sigma: X4126) 60% glycerol 60 mM EDTA.	1 tube with 175 µl
Agarose	3% in 1X TAE or 1X TBE	3 g
Gel Red Staining dye	10,000 X (Biotium #41003)	25 µl
DNA ladder	(Fermentas: #SM1343)	6 µl
TAE	50X: 242 g Tris Base 57.1 ml glacial Acetic Acid 18.6 g EDTA 10X: volume to 1 L of distilled water.	Dilute 1:49 in distilled water before using. Need several liters.
TBE	108 g Tris Base 55 g Boric Acid 9.3 g EDTA 10X volume to 1 L of distilled water.	Dilute 1:9 in distilled water before using. Need several liters.
Tris, pH 8.0	1M: 121.1 g Tris base 700 ml distilled water pH to 8.0 with HCl Bring volume to 1 L	

Materials

Pipettemen (1 set for every 5 students) 500 µl fixed volume pipetteman or P1000 200 µl pipetteman (set at 180 µl), or P200 20 µl pipetteman, or P20
Pipette tips (for P1000, P200, and P20)
1.5 ml centrifuge tubes (100) 0.5 ml centrifuge tubes (25)
DNA extraction swabs (25) Catch-All Collection Swab Catalog # QEC87100 Epicentre Biotechnologies.
2 Table-top Centrifuges 14,000 rpm
Beaker of blue stained water for students to practice for pipetting.
Heating block/water bath at 95˚C then turned to 37°C for week 2
Bucket with bleach for disposal of swabs
PCR thermocycler

Notes for the Instructor

Demonstration of Pipetting

http://www.youtube.com/watch?v=tL0acTneiNY&feature=PlayList&p=1412854125AC804B&playnext=1&playnext_from=PL&index=3

DNA Dilution Buffer:

In a single 1.5 ml tube, you will mix the following: (enough for 25 reactions)

	25 reactions	(equals single reaction)
Dilution Buffer	500 µl	(20 µl)
DNA Release Additive	12.5µl	(0.5 µl)

Place 100 µl aliquots into tubes and place the (DIL) on the top and place two in an ice bucket per bench.

PCR Reaction Mix

	25 reactions	(equals single reaction)
2x PHIRE Animal Tissue Buffer	650 µl	(25 µl)
Primer (forward, PF, 10 µM)	25 µl	(1.0 µl)
Primer (reverse, PR, 10 µM)	25 µl	(1.0 µl)
Sterile Water	525 µl	(21 µl)
PHIRE Hotstart 2 DNA Polymerase	25 µl	(1 µl)

Cap and flick tube with finger to mix and place in ice bucket for instructors

- At lab time, instructors will pipette 49 µl of reaction mix to each of the 21 (0.5 µl) tubes in the ice bucket (20 from students, one control water.)
- Instructors will also add 1 µl of sterile water to the control 0.5 µl tube.
- Using a new pipette each time, students will add 1 µl from their 1/10 dilution tube to the 0.2 ml tube in the ice bucket.
- Place 0.5 µl tubes into the PCR machine and start the program.

PCR Program

Initial Denaturation 98˚C 5 min
30 cycles of
98˚ C for 5 sec
67˚C for 5 sec
72˚C for 20 sec
Final extension for 72˚C for 1 minute.

PCR Primers

(Stored in freezer undiluted, but also diluted to 10 µM to use in lab)
*Alu*Yb8NBC225F
5'-GAGTCCAGCCCATTTTAGCATGGGACA-3'
*Alu*Yb8NBC225R
5'-CCCAGCACAAACATGTCATT-3'

Restriction Digestion

Check the temperature on the 37°C heating block.
Gather the 25 newly-labeled 1.5 ml tubes from each student and place them into an ice bucket along with one 1.5 ml control tube.

Restriction Enzyme Digestion Mix

In a single 1.5 ml tube on ice, you will mix the following: (enough for 25 digestions.)

	25 digestions	(equals single digestion)
10x Buffer	91 µl	(3.5 µl)
Enzyme	26 µl	(1.0 µl)

Cap and flick tube with finger to mix
Using the P20 pipette and a single yellow tip, pipette 4.7 µl of digestion mix to each of the 25 (1.5 ml) tubes in the ice bucket (25 from students)
Students add 30 µl of PCR reaction and place at 37°C.

Gel is 3% agarose with Gel Red added. After adding loading buffer, load the gel with one lane for un-cut control (25 µl) and a second lane for digested DNA (37 µl). Run gel at 150 constant voltage for about an hour.

Answer Key for Pre-Lab for Week 1

1. **Context**:

   a. Imagine you are at a party and someone pipes up, "But of course, <insert ethnic group> are < choose characteristic, smarter than, more athletic, more thrifty, more hard-working> than <insert other ethnic group>; it's in their genes." Using what you read in the article, how would you explain what is known about the utility of social definition of race, such as skin color or other physical features, and the underlying DNA evidence of how closely related two human beings are.

   Answer: There are distinct differences between ethnic groups that have arisen in the past 100,000 years since humans migrated out of Africa into the rest of the world. These differences can be seen in changes to our DNA, called polymorphisms. Some polymorphisms do occur in genes, and they can contribute to individual variation in traits and genetic diseases.

   b. According to the authors, why would it be useful to define race from a biological standpoint?

   Answer: Common definitions of race do sometimes work well to divide groups according to genetically determined propensities for certain diseases, or for how people respond to drug treatments.

   Give an example used in the article of knowledge of racial differences being used to benefit humans.

   Answer: Several polymorphisms in CCR5 prevent HIV infection or influence the rate at which HIV-1 infection leads to AIDS and death. One polymorphism, for example, is associated with delayed disease progression in European-Americans but accelerated disease in African-Americans. Researchers can only study such population-specific effects--and use that knowledge to direct therapy--if they can sort people into groups.

   c. In the article, what reasons are given to continue using self-reported ancestry as a diagnostic tool for physicians rather than using genetic testing?

Answer: Genetic screening is expensive, inefficient, and raises concerns about privacy and consent. In addition, for diseases influenced by the cumulative results of polymorphisms in several genes such as hypertension and diabetes, recent research suggests that polymorphisms that have a particular effect on one group may have a different effect on another group. This kind of complexity would make it much more difficult to use detected polymorphisms as a guide to therapy. Until further studies are done on the genetic and environmental contributions to complex diseases, physicians may have to rely on information about an individual's ancestry to know how best to treat some diseases.

d. What is the function of *Alus* in the human genome, and what characteristic of transposons makes them perfect tracers for genealogy?

Answer: *Alu*s are short pieces of DNA that are similar in sequence to one another but have no real function in the human genome except as parasites. *Alu*s replicate occasionally, and the resulting copy splices itself at random into a new position on the original chromosome or on another chromosome, usually in a location that has no effect on the functioning of nearby genes. *Alu*s are perfect tracers for geneology because each insertion is a unique event. Once an *Alu* sequence inserts itself, it can remain in place for eons, getting passed from one person to his or her descendants. Therefore, if two people have the same *Alu* sequence at the same spot in their genome, they must be descended from a common ancestor who gave them that specific segment of DNA.

e. Of the total genetic variability within the human species, approximately what percentage of variation can be found within a population, say in Europe? How much variation exists between populations, say Europeans as compared to Asians?

Answer: "Many studies have demonstrated that roughly 90% of human genetic variation occurs within a population living on a given continent, whereas about 10% of variation distinguishes continental populations. In other words, individuals from different populations are, on average, just slightly more different from one another than are individuals from the same population.

2. **Method**

In your own words, define a polymorphism. Use two made-up DNA sequences to help support your definition.

Explain how researchers could analyze polymorphisms such as *Alus* to determine ancestry.

Answer: Researchers have found that a specific *Alu* polymorphism, for example, one on chromosome 1 occurs in roughly 95 percent of sub-Saharan Africans who have been sampled, 75 percent of Europeans and northern Africans, and 60 percent of Asians, whereas a different *Alu* polymorphism on chromosome 7 is carried by approximately 5 percent of sub-Saharan Africans, 50 percent of Europeans and northern Africans, and 50 percent of Asians. Some individuals carry both polymorphisms. No single polymorphism can, by itself, distinguish all the members of one major human group from all the members of another group, but by analyzing hundreds of these polymorphisms, scientists can group individuals sampled from different locations on the basis of their genetic profiles.

3. **Results**

Give two examples of how researchers have documented polymorphisms that result in effects on health.

Answer: The mutations responsible for sickle cell disease and some cases of cystic fibrosis, for instance, result from genetic changes that appear to have risen in frequency because they were protective against diseases prevalent in Africa and Europe, respectively. People who inherit one copy of the sickle cell polymorphism show some resistance to malaria; those with one copy of the cystic fibrosis trait may be less prone to the dehydration resulting from cholera. The symptoms of these diseases arise only in the unfortunate individuals who inherit two copies of the mutations. Genetic variation also plays a role in individual susceptibility to one of the worst scourges of our age: AIDS. Some people have a small deletion in both their copies of a gene that encodes a particular cell-surface receptor called a chemokine receptor 5 (CCR5). As a result, these individuals fail to produce CCR5 receptors on the surface of their cells. Most strains of HIV-1, the virus that causes AIDS, bind to the CCR5 receptor to gain entry to cells, so people who lack CCR5 receptors are resistant to HIV-1 infection. This polymorphism in the CCR5 receptor gene is found almost exclusively in groups from northeastern Europe.

Several polymorphisms in CCR5 do not prevent infection but instead influence the rate at which HIV-1 infection leads to AIDS and death. Some of these polymorphisms have similar effects in different populations; others only alter the speed of disease progression in selected groups. One polymorphism, for example, is associated with delayed disease progression in European-

Americans but accelerated disease in African-Americans. Researchers can only study such population-specific effects--and use that knowledge to direct therapy--if they can sort people into groups.

You just learned in last week's lab that *Alu* transposable elements are useful as DNA markers for human population studies. In fact, there are over 5000-7000 new *Alu* insertions into the human genome since our ancestors split from a common ancestor with chimpanzees. Some of these new insertions may have accumulated mutations that result in polymorphisms that can be used as markers to identify ancestry. Researchers David H. Kass, Nicole Jamison, Melanie M. Mayberry, and Eillen Tecle (Kass, Jamison, Mayberry, & Tecle, 2007) identified a single *Alu* element (YB8) that exists in human populations as 3 different polymorphisms. These polymorphisms are changes in the DNA sequence that must have occurred accidentally in different human populations since the time they migrated to different global regions. They sampled populations from Africa, South America, Europe, and Asia for these polymorphisms and uncovered variations in the presence of the different alleles that could be used to elucidate ancestry. The PCR primers that you used last week amplified that *Alu*. This week you will conduct a restriction enzyme digestion of your *Alu* elements that will allow you to identify which polymorphisms you inherited from you ancestors.

Answer Key for Pre-Lab for Week 2

1. **Context**:

   The nucleotide sequence of the YB8 *Alu* element that you will amplified last week in lab can be found in human populations with one of the following three polymorphisms (S1, S2, and L).

   Answer: See annotated alignment below.

b. Assuming that these polymorphisms occurred following random genetic changes (mutations to the original sequence (YB8). How many changes occurred in each??

Polymorphism	# Nucleotide Changes
YB8 » S1	8
YB8 » S2	9
YB8 » L	10

```
    62     70 73
YB8 _GGCCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGTGGATCATGAGGTCA
S1  T.....GGCGCC.............................................................
S2  T.......T..A.............................................................
L   T.......T..A.............................................................

                                          172
YB8 GGAGATCGAGACCATCCTGGCTAACAAGGTGAAACCCCGTCTCTACTAAAAATACAAAAAATTAGCCGGGCGC
S1  ....................................A.....................................
S2  .........................................................................
L   .........................................................................

            217                236-237       250    257
YB8 GGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGCGTGAACCCGGGAAGCGG
S1  ............................TA............G......G.....................
S2  ............................TA............G......G.....................
L   ..........A.................TA............G......G.....................

                       298                                         338
YB8 AGCTTGCAGTGAGCCGAGATTGCGCCACTGCAGTCCGCAGTCCGGCCTGGGCGACAGAGCGAGACTCCGTCTC
S1  ...................C.........................................................
S2  ...................C.........................................A.............
L   ...................C.........................................A.............
```

c. The changes in the *Alu* element above can be thought of with a plagiarism analogy: Imagine a professor gets 4 nearly identical responses to an in-class writing assignment, and he wishes to figure out what evidence in the writing may uncover the story of what occurred. One student, John, submits a nearly perfect paper. The second student, James, submitted a nearly identical paper to John's but he has a missed word, number 300, that renders the text meaningless at that point. The third student, Julie, submitted a paper identical to James' with the same mistake at word 300, but she also has a new mistake at word 1100. Finally, the fourth student, Susan, has the same identical error at word 300, but she has a new mistake at word 124. In this scenario, the professor could try to determine the order of the plagiarism. Obviously, the papers were all copied in some way, and one would have to assume that the introduction of errors occurred during the copying. So, the professor can start to chart out (as shown below) what might have occurred before he confronts the students:

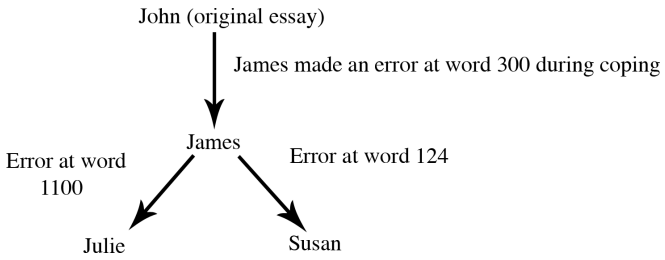

Figure 1. Plagiarism Analogy. The professor could match the changes in each essay that then see if this pattern was supported by their seating arrangements.

Answer: This is exactly how scientists compare DNA sequence changes in these polymorphisms. Random errors must have occurred in the copying mechanism during DNA replication, and the descendents of those humans would have inherited the errors. One can then use the errors like the professor did to chart the most logical series of steps. Using the YB8, S1, S2, and L sequences construct a map like the one above that charts the changes in the evolution of the original YB8 element in human populations. (Be sure to mark your chart with each of the four elements and describe each mutational change..

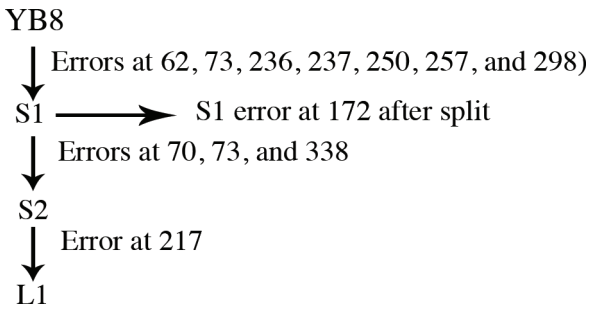

Figure 1b. Plagiarism Analogy (with answers). The professor could match the changes in each essay that then see if this pattern was supported by their seating arrangements

2. **Method**

The nucleotide changes in the four different polymorphisms for this *Alu* allele can be distinguished by DNA sequencing, so one could determine which two alleles you had simply by sending your PCR reactions off to be sequenced. However, there is a way that you can determine which polymorphism you have using two techniques, restriction enzyme digestion and gel electrophoresis that we can do cheaply in this laboratory. You'll remember that restriction enzymes are able to cut DNA into pieces at very specific sequences. For example, the restriction enzyme KasI recognizes the sequence GGCGCC underlined in the sequence from homework question #1. Using the sequence from question #1, fill in the following table to indicate how many times the enzyme would cut each polymorphism allele S1, S2, and L, and how many pieces would be generated.

Table 2. Number of enzyme cleavage points and size of restriction fragments generated.

Polymorphism	**# Times Enzyme would cut**	**# Pieces Made**
S1	2	3
S2	1	2
L	0	1

3. **Results**

Using the following figures of the YB8, S1, S2, and L *Alu* polymorphisms that were amplified by PCR last week, mark the location of the KasI sites with a horizontal line and indicate the nucleotide number, so you can visualize your answers from above. (*4 points*)

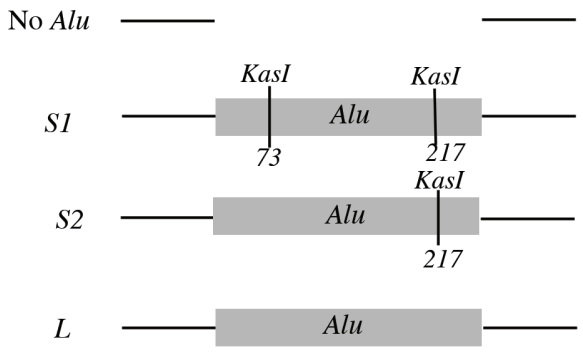

Figure 2. Location of KasI sites in the Four *Alu* Polymorphisms

Answer Key for Restriction Experimental Designs for Week 2

Go back to your answers to question 3 from the pre-lab, for each allele (S1, S2, and L) indicate the sizes of the DNA fragments that would be generated from restriction enzyme digestion of each allele.

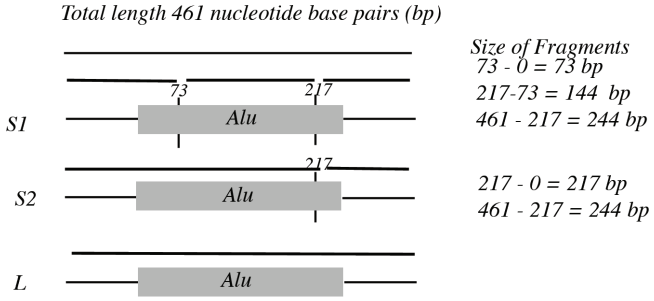

Figure 3. (With answers). Restriction fragments generated from the three alleles. Using these lengths and considering a person with the L allele on one chromosome and the S1 allele on the other chromosome (L/S1 genotype), indicate the number and size of all fragments created by KasI digestion of their PCR sample.

So, using these lengths a person with the L allele on one chromosome and the S1 allele on the other chromosome (L/S1 genotype). Indicate the number and size of all fragments created by KasI digestion of their PCR sample:

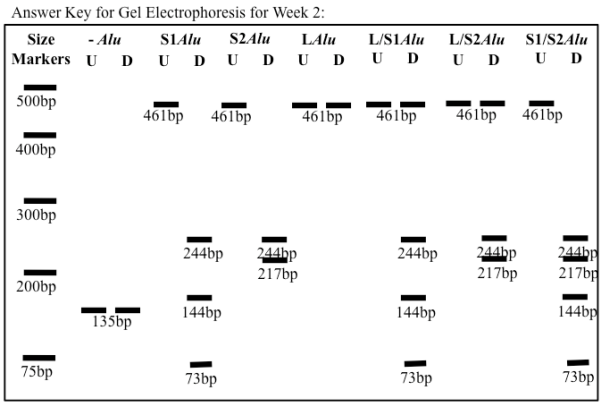

Figure 4. (With answers). Gel Electrophoresis of the PCR fragments generated. Using just the ends of the sequence without the *Alu* element is 135 bp and is shown in the second lane. Fill in the expected sizes for the restriction digests of the remaining *Alu* elements. (U indicates undigested, and D, digested.)

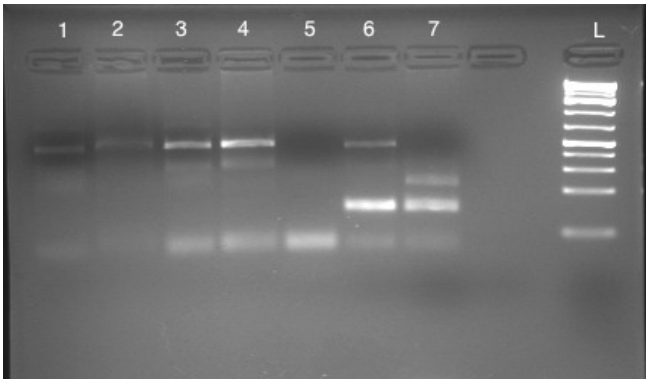

Figure 5. Sample Results of Restriction Digests of PCR Products. Three individuals are shown (lanes 1 and 2, 3 and 4, 6 and 7). Lanes 1, 3, and 6 are undigested PCR results. Lanes 2, 4, and 7 are the results after digestion with BanI. Lane 5 is a negative control. In lane size a large band and small band is present showing the student is heterozygous. In lane 7 the large band was cut into a smaller band. Based on the sizes it suggests that it is an S2 allele and the genotype is S2/-. Lanes 2 and 4 did not digest suggesting a L allele and the students are L/L. All students were Caucasian.

Dot Sheet

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We want to thank Christi Bradshaw for help developing the procedure and Kristen Miller for helpful comments on the protocol and teaching materials. This work was funded by a Howard Hughes Medical Institute Professor Grant (Grant number 52005731) to Susan R. Wessler.

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Kass, D. H., Jamison, N., Mayberry, M. M., & Tecle, E. 2007. Identification of a unique *Alu*-based polymorphism and its use in human population studies. *Gene* 390(1-2): 146-152.

About the Authors

Peggy Brickman received her bachelor's degree in biology from Columbia College in 1987, and her Ph.D. in genetics from U.C. Berkeley in 1993. She is an associate professor of plant biology at the University of Georgia where she teaches non-majors introductory biology to approximately 650 students each semester. She is currently researching the scientific literacy gains made by students in general education biology courses and has been actively involved in developing a number of case studies for teaching basic biology content. She is also actively involved in future faculty development through courses and workshops she offers to graduate students and post-docs.

Jim Burnette received his BS in Biology from The University of Georgia and his Ph.D. in the Biological Sciences from Carnegie Mellon University (Pittsburgh, PA). He has developed and taught research intensive lab courses at Carnegie Mellon and the University of Georgia. Currently Jim is an Academic Coordinator and Professional Researcher at the University of California Riverside where he will develop a research intensive program for first year students. Jim is most interested in translating projects from research laboratories into lab course projects in the areas of genomics and bioinformatics.

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