Teacher Prep & Follow-up

STUDENT LAB BRIEFING

To get the most out of the laboratory experience, we strongly suggest that you prepare your class prior to coming to the DNA Learning Center. Make sure your students have a basic understanding of the structure and function of DNA. Notions of heredity, polymorphism and transposable DNA elements will also be useful although not essential. They should read the enclosed **Carolina Tips** article, "Polymerase Chain Reaction." After reading this article, students should be able to discuss:

- The mechanism and applications of polymerase chain reaction.
- Use of electrophoresis and agarose to separate DNA fragments.

You may also wish to have students read over the lab theory and protocol accessible online at: http://www.geneticorigins.org/pv92/aluframeset.htm.

AT THE DNA LEARNING CENTER

Before starting the experiment, the instructor will briefly review the purpose of the laboratory and the techniques involved. Students will be introduced to the lab equipment. The lab protocol will be discussed step by step. Students will have 45 minutes during the amplification step to eat their lunch/snack if they wish. Teachers must supervise students in the lunchroom, or students may choose to eat on the school bus if available.

RESULTS AND DISCUSSION

In addition to the questions in the lab protocol available online, you can discuss the ethical ramifications of DNA fingerprinting and genetic screening, the Human Genome Project, and genetic engineering. There are often articles in journals on teaching bioethics, for example "Genetic Engineering—A Lesson on Bioethics for the Classroom," by Kerri Armstrong and Kurt Weber (*The American Biology Teacher*, May 1991, Volume 53(5)). There is also a very good section on societal issues in *A Sourcebook of Biotechnology Activities* from the National Association of Biology Teachers and the North Carolina Biotechnology Center.

Answers to some of the online questions are in bold.
(Follow-up activities and questions here: http://www.geneticorigins.org/pv92/aluframeset.htm)

I.-3. What can you say about any person in the class who has at least one "+" allele?

They are related to the original common ancestor in which the *Alu* first jumped into the PV92 locus. They have all inherited an identical allele from this common ancestor. This is identity by descent.

II.-1. What is the p-value for your Chi-Square? Is it less than .05? If so, can you suggest any factors that might account for why your observed population is not in Hardy-Weinberg equilibrium?

Hardy-Weinberg equilibrium exists only when population size is large, mating is random, all genotypes survive equally well, there is no immigration, and no mutation occurs. If any of these criteria are violated, then a population will not remain in Hardy-Weinberg equilibrium over time.

III.-1. Based on the results you recorded, how useful is the PV-92 *Alu* polymorphism in distinguishing populations from each other? Why do you think that the PV-92 allele frequencies differ significantly between some populations, while not between others? Do you think you could use PV-92 data to answer the questions of where humans originated and the paths by which they spread throughout the world? What other population data sets or types of information might you need to accurately answer this question?

If two populations are genetically distinct (i.e., no gene exchange between the two populations has occurred for long period of time), then *Alu* insertion rates may differ significantly. Because of this limited information content (two alleles, three genotypes), *Alu* polymorphisms alone would not provide enough information to accurately reconstruct human origins. This shortcoming could be overcome by including additional *Alu* polymorphisms in the analysis.

III.-2. Use the Heterozygosity Feature of the Analyze Function to determine the + allele frequency in a number of populations representing differing parts of the world. Select 'Heterozygosity' from the pulldown menu on the RIGHT. Click the round checkbox underneath to select a group for analysis. (You can only analyze one group at a time). Print the world map (http://www.bioservers.org/map.html) and plot the + allele frequencies on it.

a. Do you notice any pattern in the allele frequencies?
b. Suggest a hypothesis about the origin and dispersal of the *Alu* allele that accounts for your observation.
c. Which of these mechanisms is consistent with the statistical evidence that PV92 first inserted about 200,000 years ago?

a. The + allele frequency shows a distinct east-to-west cline. The + allele frequency is greatest in Southeast Asia and much lower in Africa, Europe, and the Americas. Intermediate frequencies are found in India.

b.
- **The original PV92 insertion arose in Southeast Asia and then dispersed to other regions.**
- **The original PV92 insertion arose in an early group of modern humans in Africa. This group then split to give rise to groups that left Africa and migrated to Europe and Asia. The allele frequency drifted randomly in these groups and stabilized at different levels when the populations grew and reached Hardy-Weinberg equilibrium.**
- **The PV92 insertion arose in Africa and drifted to a frequency of about 15-20%. By chance, the founders of some Asian populations had a much higher allele frequency. This is called the "founder effect". The higher frequency stabilized when the population reached equilibrium.**
- **The process of "gene flow," where genes are exchanged by mating between adjacent populations, may have also contributed to the cline.**

c. Only the last three.

III.-3. Considering your results, do you think this protocol could be used forensically to link a suspect with a crime?

No, for two major reasons. First, hair is rarely used as a DNA source in forensic cases, because the vast majority of hairs found at a crime scene do not have root or sheaths. Second, the *Alu* insertion system has only two alleles. Forensic testing uses repeat polymorphisms, such as VNTRs and STRs, which have tens to hundreds of alleles. Also, around thirteen markers on different chromosomes are now examined.

III.-4. *Alu* can be considered parasites of retroviruses, which produce the enzyme reverse transcriptase needed for transposition. Why is this so? Propose a transposition mechanism requiring reverse transcriptase.

Alu elements have no protein coding sequence, and are not capable of producing any enzymes that might be required in their life cycle. Most _Alu_s are found within introns, and are transcribed into RNA during transcription of their host genes. Reverse transcriptase then converts the _Alu_ RNA into a double-stranded DNA element, which is capable of insertion.

III.-5. An *Alu* insertion has only two states: + and -. How does this relate to information stored in digital form by a computer? How much digital information is provided by an *Alu* genotype?

Alu +/- is equivalent to a digital 0/1, or one bit of information. An _Alu_ genotype (++, --, or +-) contains two bits of information.

IV.-1. Examine the graph that shows the frequency of the "+" allele versus generation number. In general, what happens to the "+" allele (i.e., the *Alu* insertion) over time? Are there any runs in which the *Alu* insertion begins to spread throughout the population during the first 100 generations? Is this a common or rare occurrence? For the runs where the *Alu* allele is not lost from the population, what happens to the allele frequency when the population size "explodes" (generation 101-200 in Population 2)? Conversely, what happens to the *Alu* allele when the population is kept small (generation 101-200 in Population 3)? In addition to population size, can you think of any other factors that would help an *Alu* insertion event spread? (Hint: What would happen if inheriting an *Alu* insertion conferred a selective advantage to the individual carrying the insertion?)

In the majority of runs, the + allele is estimated from the population over a number of generations. Occasionally, the _Alu_ insertion does spread throughout the population, sometimes even becoming fixed (reaching + allele frequency of 1, or 100%). In small populations, the probability of + allele fixation is equal to the initial + allele frequency. With increasing population size, the probability of gene fixation decreases. Selective advantage conferred to those individuals carrying an _Alu_ insertion would increase the spread of this marker throughout a population, and the probability of eventual fixation.