

## INFORMATION FOR INSTRUCTOR

### CONCEPTS AND METHODS

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This laboratory can help students understand several important concepts of modern biology:

- How to collect and analyze genetic information in populations.
- The use of allele and genotype frequencies to test Hardy-Weinberg equilibrium.
- The use of DNA polymorphisms in the study of human evolution.
- Identity by descent from a common ancestor.
- The movement between *in vitro* experimentation and *in silico* computation.

The laboratory uses several methods for modern biological research:

- DNA extraction and purification.
- Polymerase chain reaction (PCR).
- Gel electrophoresis.
- Bioinformatics.

### LAB SAFETY

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The National Association of Biology Teachers recognizes the importance of laboratory activities using human body samples and has developed safety guidelines to minimize the risk of transmitting serious disease. ("The Use of Human Body Fluids and Tissue Products in Biology," *News & Views*, June 1996.) These are summarized below:

- Collect samples only from students under your direct supervision.
- Do not use samples brought from home or obtained from an unknown source.
- Do not collect samples from students who are obviously ill or are known to have a serious communicable disease.
- Have students wear proper safety apparel: latex or plastic gloves, safety glasses or goggles, and lab coat or apron.
- Supernatants and samples may be disposed of in public sewers (down lab drains).
- Have students wash their hands at the end of the lab period.
- Do not store samples in a refrigerator or freezer used for food.

The risk of spreading an infectious agent by this lab method is much less likely than from natural atomizing processes, such as coughing or sneezing. Several elements further minimize any risk of spreading an infectious agent that might be present in mouthwash samples:

- Each experimenter works only with his or her sample.
- The sample is sterilized during a 10-minute boiling step.
- There is no culturing of the samples that might allow growth of pathogens.
- Samples and plasticware are discarded after the experiment.

## INFORMED CONSENT AND DISCLOSURE

Student participation in this experiment raises real-life questions about the use of personal genetic data: What is my DNA sample being used for? Does my DNA type tell me anything about my life or health? Can my data be linked personally to me?

There is consensus that a human DNA sample should be obtained only with the willing consent of a donor, who understands the purpose for which it is being collected. Thus, this experiment should be explained ahead of time and students given the option to refrain from participating. (Some teachers may wish to have parents sign a consent form, such as those filled out for a field trip.) There is also consensus that a DNA sample be used only for the express purpose for which it is collected. Thus, student DNA samples should be thrown away after completing the experiment.

The PV92 polymorphism was specifically selected for this experiment because it is phenotypically neutral—it has no known relationship to any trait, disease state, or sex determination.

PV92 alleles are inherited in a Mendelian fashion and can give indications about family relationships. To avoid the possibility of suggesting inconsistent inheritance, it is best not to generate genotypes from parent-child pairs. In any event, this two-allele system would be less likely to turn up an inconsistency than the ABO blood groups. Furthermore, the chance that student samples can be mixed up when isolating DNA, setting up PCR reactions, and loading electrophoresis gels provides no certainty to any of the genotypes obtained in the experiment. (A forensic laboratory would use approved methods for maintaining “chain of custody” of samples and for tracking samples.)

## INSTRUCTOR PLANNING, PREPARATION, AND LAB FINE POINTS

The following table will help you to plan and integrate the four parts of the experiment.

Part	Day	Time	Activity
I. Isolate DNA	1	60 min.	<b>Pre-lab:</b> Prepare and aliquot saline solution Prepare and aliquot 10% Chelex® Aliquot proteinase K (alternate) Make centrifuge adapters Set up student stations
		30 min.	<b>Lab:</b> Isolate student DNA
II. Amplify DNA by PCR	1	15 min. 15 min. 60–150 min.	<b>Pre-lab:</b> Aliquot PV92B primer/loading dye mix <b>Lab:</b> Set up PCR reactions <b>Post-lab:</b> Amplify DNA in thermal cycler
III. Analyze PCR Products by Gel Electrophoresis	2	15 min. 30 min.	<b>Pre-lab:</b> Dilute TBE electrophoresis buffer <b>Lab:</b> Prepare agarose gel solution and cast gels. Load DNA samples into gel Electrophorese samples
	3	15 min. 20+ min. 20+ min. 30–45 min. to overnight 20 min.	<b>Post-lab:</b> Stain gels De-stain gels (for CarolinaBLU™) Photograph gels
Results and Discussion	4	30-60 min.	Score PV92 genotypes; determine class genotype and allele frequencies

## I. ISOLATE DNA FROM CHEEK CELLS

Saline mouthwash is the most reproducible of the simple methods to obtain human DNA for PCR. The mouthwash gently loosens a large number of single cells and small clusters of cheek cells. This maximizes the surface area of cells, allowing for virtually complete lysis during boiling. Cheek brushes and swabs generally yield larger clumps of cells, which are less effectively lysed by boiling.

With careful lab management, up to 90% of students should be able to “score” their *Alu* genotypes using the mouthwash method. Be especially watchful after the initial centrifugation step. Most students will have compact pellets that stay attached to the tube when the supernatant is poured off. However, about 10% of students will have diffuse or slimy masses that do not pellet well. Centrifuge these samples again, then carefully pipet out as much supernatant as possible. Surprisingly, food particles rinsed out with the mouthwash have little effect on PCR amplification. Still, it is best to avoid eating before the experiment, because food particles, especially from fruits, may block the pipet tip and make pipetting difficult.

It is worth a diversion to allow students to view their own squamous epithelial cells under a compound microscope. Add several  $\mu\text{L}$  of suspension remaining after Step I. 8. to a microscope slide, add a drop of 1% methylene blue (or other stain), and add a cover slip.

DNA is liberated from cheek cells by boiling in 10% Chelex<sup>®</sup>, which binds contaminating metal ions that are the major inhibitors of PCR. The boiling step is most easily accomplished using the same thermal cycler used for PCR. To do this, provide each student with 100  $\mu\text{L}$  of 10% Chelex<sup>®</sup> suspension in a PCR tube that is compatible with the thermal cycler you will be using: either 0.2 mL or 0.5 mL. It is not necessary to use a “thin-walled” tube. Alternatively, use 1.5-mL tubes in a heat block or a boiling water bath. *Watch out for lids opening as the tubes heat.* (Make a simple water bath by maintaining a beaker of water at a low boil on a hot plate. Place 1.5-mL tubes in a floating rack or in holes punched in a double layer of aluminum foil over the top. If using aluminum foil, insure that tubes are immersed, and add hot water as necessary to maintain water level.)

### **Pre-lab Preparation**

Prepare saline by dissolving 0.9 g NaCl in 100 mL distilled or deionized water. For each student, aliquot 10 mL into a 15-mL polypropylene tube.

Prepare 10% Chelex<sup>®</sup> by adding 15 mL distilled or deionized water to 1.5 g of Chelex<sup>®</sup>. For each student, aliquot 100  $\mu\text{L}$  of 10% Chelex<sup>®</sup> into either a 0.2-mL or 0.5-mL tube (whichever format is accommodated by your thermal cycler). Alternatively, use a 1.5-mL microcentrifuge tube if you are planning to use a heat block or water bath instead of a thermal cycler. *The Chelex<sup>®</sup> resin quickly settles, so be sure to shake the stock tube to re-suspend the Chelex<sup>®</sup> each time before pipetting a student aliquot.*

Remove caps from 1.5-mL tubes to use as adapters in which to centrifuge the 0.5-mL PCR tubes used for Chelex<sup>®</sup> extraction. Two adapters are needed to spin 0.2-mL PCR tubes—a capless 0.5-mL PCR tube is nested within a capless 1.5-mL tube.

### **Pre-lab Set Up for DNA Isolation from Cheek Cells** (per student station)

- Saline solution (0.9% NaCl) tubes, 10 mL (in 15 mL tube)
- 10% Chelex<sup>®</sup>, 100  $\mu\text{L}$  (in 0.2 or 0.5 mL tube, depending on thermal cycler)
- 2 1.5-mL microcentrifuge tubes
- Permanent marker
- Micropipets and tips (10–1,000  $\mu\text{L}$ )
- Microcentrifuge tube rack
- Container with cracked or crushed ice
- Paper cup

## Shared Items

Microcentrifuge  
Microcentrifuge adapters for 0.2-mL or 0.5-mL PCR tubes  
Thermal cycler  
Vortexer (optional)

## I. (ALTERNATE) ISOLATE DNA FROM HAIR SHEATHS

Hair roots provide the simplest source of DNA for PCR amplification; no special equipment is required for extraction. Hairs also are an extremely safe source of cells. Risk of spreading an infectious agent is minimized by "dry" collection, which does not involve any body fluid or generate any supernatant. This method also stresses the power of PCR in forensic cases—even one growing hair root provides enough DNA for excellent amplification.

HOWEVER, forensic biologists generally rate hair as a poor source of DNA for analysis, for the same reason that it can prove difficult in the classroom. Most plucked or shed hairs are broken off from the root, which is the source of cells for DNA extraction.

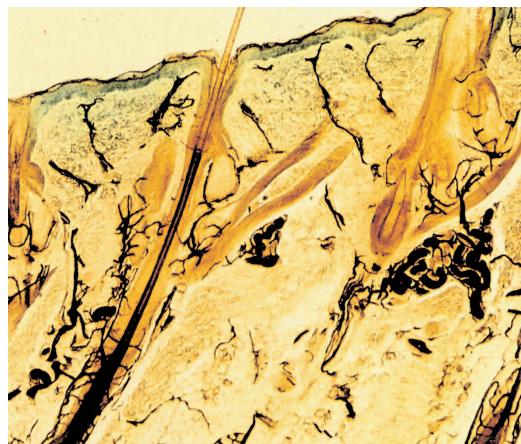
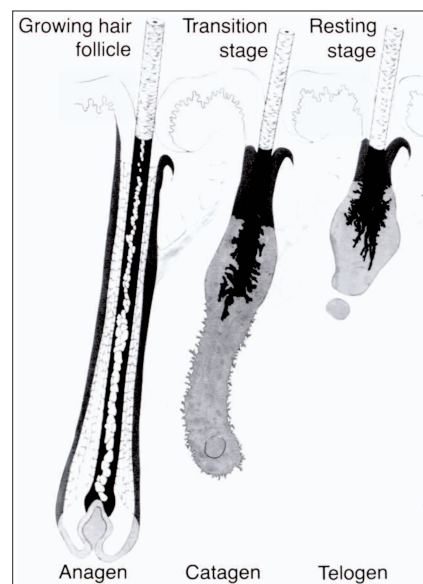
The success of this method is entirely dependent upon finding large roots from growing hairs. This can be tricky and time consuming—if often hilarious. With vigilance, up to 80% of students may find hairs with good roots from which to isolate DNA. However, it is more likely that only about 60–70% of students ultimately will be able to score their *Alu* genotypes using this method.

A hair is anchored in the skin by a follicle, or "root," whose growing cells produce the hair shaft. Hair goes through a growth cycle with alternating periods of growth and quiescence during which the follicle increases and decreases in size. During the growth phase, the follicle extends up the hair shaft in a structure called the sheath. The sheath is a rich source of cells. The sheath membrane is easily digested by treatment with proteinase K, releasing squamous cells singly or in small clusters. A high percentage of these cells are lysed by boiling and release DNA.

The sheath decreases in size as the hair follicle enters a resting stage (see drawing and micrograph of growing and resting follicles). The withered bulb of a resting follicle is, in fact, what most people would consider a "root." Resting follicles usually yield little DNA for analysis. First, there are fewer cells. Second, proteinase K treatment does not effectively digest the shriveled root mass, and only cells at the edge are lysed by boiling.

Successful amplification of the PV92 locus, which is available in only two copies per cell, is closely correlated to presence of a sheath on the hair shaft. One or two hairs with long sheaths will provide plenty of DNA for PCR amplification. Three or four good sized roots will usually work, especially if they have at least small sheaths.

A good sheath is unmistakable. Especially contrasted on a dark hair, it glistens when held up to the light and extends several mm up the hair shaft. Make sure to show off the first several



good sheaths that turn up, so other students will know what to look for. Because of the hair growth cycle, most people find sheaths only on some hairs. Students whose hair grows slowly may have difficulty finding sheaths, and thin or brittle hair is likely to break off before the root. If students are having difficulty finding sheaths on hairs pulled from their scalps, have them try hairs from the eyebrow or arm.

Sheaths are the most underrated source of squamous cells for microscopic examination. Give them a try! Simply place a sheath on a microscope slide and add a drop of proteinase K (100 mg/mL). Let stand for several minutes, to allow the proteinase K to digest the sheath membrane. Then add a drop of methylene blue or other cell stain, add a cover slip, and gently press to disrupt the sheath membrane. Observe under medium power and at several time points, to see the effect of enzyme digestion. If you gently press the cover slip while the slide is on the microscope stage, you should be able to observe squamous cells squirting out of tears in the sheath membrane.

### **Prelab Preparation**

For each student, aliquot 100  $\mu$ L of 100 mg/mL proteinase K into either a 0.2-mL or 0.5-mL tube (whichever format is accommodated by your thermal cycler). Alternatively, use a 1.5-mL microcentrifuge tube if you are planning to use a heat block or water bath instead of a thermal cycler.

#### **Pre-lab Set Up for DNA Isolation from Hair Sheaths** (per student station)

- 100 mg/mL proteinase K, 100  $\mu$ L (in 0.2- or 0.5-mL PCR tube)
- Permanent marker
- Scalpel or razor blade
- Forceps or tweezers

#### **Shared Items**

- Thermal cycler (or water bath or heat block)
- Container with cracked or crushed ice
- Vortexer (optional)

## **II. AMPLIFY DNA BY PCR**

The primer/loading dye mix incorporates the appropriate primer pair (0.26 picomoles/ $\mu$ L of each primer), 13.8% sucrose, and 0.0081% cresol red. The inclusion of the loading dye components, sucrose and cresol red, allows the amplified product to be directly loaded into an agarose gel for electrophoresis. Each Ready-To-Go™ PCR Bead contains reagents so that when brought to a final volume of 25  $\mu$ L, the reaction contains 2.5 units of *Taq* DNA polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, and 200  $\mu$ M of each dNTP.

The lyophilized *Taq* DNA polymerase in the bead becomes active immediately upon addition of the primer/loading dye mix and template DNA. In the absence of thermal cycling, “nonspecific priming” at room temperature allows the polymerase to begin generating erroneous products, which can show up as extra bands in gel analysis. *Therefore, work quickly. Be sure the thermal cycler is set and have all experimenters set up their PCR reactions as a coordinated effort. Add primer/loading dye mix to all reaction tubes, then add each student template, and begin thermal cycling as quickly as possible. Hold reactions on ice until all student samples are ready to load into the thermal cycler.*

PCR amplification from crude cell extracts is biochemically demanding, and requires the precision of automated thermal cycling. However, amplification of the PV92 locus is not complicated by the presence of repeated units. Therefore, the recommended amplification times and temperatures will work adequately for most common thermal cyclers, which ramp between temperatures within a single heating/cooling block. **IMPORTANT:** A different cycling profile is required for Robocycler or other brands of thermal cyclers



that physically move PCR reaction tubes between multiple temperature blocks. Because there is no ramping time between temperatures, these machines require the longer cycling times listed below:

Denaturing step:	94°C	1 minute
Annealing step:	68°C	2 minutes
Extending step:	72°C	2 minutes

### **Pre-lab Preparation**

Aliquot 25 µL of PV92B primer/loading dye mix per student. The primer/loading dye mix may collect in the tube cap during shipping; pool the reagent by spinning the tube briefly in a microcentrifuge or by sharply tapping the tube bottom on the lab bench.

#### **Pre-lab Set Up for DNA Amplification** (per student station)

- Cheek cell DNA. 2.5 µL (from Part I)
- PV92B primer/loading dye mix, 25 µL
- Ready-To-Go™ PCR beads (in 0.2-mL or 0.5-mL PCR tube)
- Permanent marker
- Micropipet and tips (1–100 µL)
- Microcentrifuge tube rack
- Container with cracked or crushed ice

#### **Shared Items**

- Mineral oil, 5 mL (depending on thermal cycler)
- Thermal cycler

## **III. ANALYZE AMPLIFIED DNA BY GEL ELECTROPHORESIS**

The cresol red and sucrose in the primer mix function as loading dye, so that amplified samples can be loaded directly into an agarose gel. This is a nice time saver. However, since it has relatively little sugar and cresol red, this loading dye is more difficult to use than typical loading dyes. So, encourage students to load carefully.

Plasmid pBR322 digested with the restriction endonuclease *Bst*NI is an inexpensive marker and produces fragments that are useful as size markers in this experiment. The size of the DNA fragments in the marker are 1,857 bp, 1,058 bp, 929 bp, 383 bp, and 121 bp. Use 20 µL of a 0.075 µg/µL stock solution of this DNA ladder per gel. Other markers or a 100-bp ladder may be substituted.

View and photograph gels as soon as possible after appropriate staining/destaining. Over time, the small-sized PCR products will diffuse through the gel and lose sharpness. Refrigeration will slow diffusion somewhat, but for best results view and photograph gels as soon as staining/destaining is complete.

### **Pre-lab Preparation**

Prepare a 1× concentration of TBE by adding 75 mL of 20× concentrated stock into 1,425 mL of deionized or distilled water. Mix thoroughly.

Prepare a 1.5% agarose solution by adding 1.5 g of agarose to 100 mL of 1× TBE in a 500-mL flask or beaker. Heat the flask or beaker in a boiling water bath (approximately 15 minutes) or in a microwave oven (approximately 4 minutes) until the agarose is completely dissolved. You should no longer see agarose particles floating in solution when the beaker is swirled. Allow the agarose to cool to approximately 60°C,

and hold at this temperature in a hot water bath. Cover beaker or flask with aluminum foil, and skim any polymerized “skin” off the top of the solution before pouring.

**Pre-lab Set Up for Gel Analysis** (per student station)

Amplified human DNA PCR products from Part III (store on ice)  
Container with cracked or crushed ice

**Shared Items**

pBR322/*Bst*NI markers, 20  $\mu$ L per row of gel (thaw and store on ice)  
1.5% agarose in 1 $\times$  TBE (hold at 60°C), 50 mL per gel  
1 $\times$  TBE buffer, 300 mL per gel  
Ethidium bromide (1  $\mu$ g/mL), 250 mL

**or**

*Carolina*BLU™ Gel & Buffer Stain, 7 mL  
*Carolina*BLU™ Final Stain, 250 mL  
Micropipet and tips (1–100  $\mu$ L)  
Microcentrifuge tube rack  
Gel electrophoresis chambers  
Power supplies  
Water bath for agarose solution (60°C)  
Latex gloves  
Staining tray  
Transilluminator with digital or instant camera (optional)

## CarolinaBLU™ STAINING

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### POST-STAINING

1. Cover the electrophoresed gel with the *CarolinaBLU*™ Final Stain and let sit for 20–30 minutes. Agitate gently (optional).
2. After staining, pour the stain back into the bottle for future use. (The stain can be used 6–8 times.)
3. Cover the gel with deionized or distilled water to destain. Chloride ions in tap water can partially remove the stain from the DNA bands and will cause the staining to fade.
4. Change the water 3 or 4 times over the course of 30–40 minutes. Agitate the gel occasionally.
5. Bands that are not immediately present will become more apparent with time and will reach their maximum visibility if the gel is left to destain overnight in just enough water to cover the gel. Gels left overnight in a large volume of water may destain too much.

### PRE-STAINING

*CarolinaBLU*™ can also be used to stain the DNA while it is being electrophoresed. Pre-staining will allow students to visualize their results prior to the end of the gel run. However, post-staining is still required for optimum viewing.

To pre-stain the gel during electrophoresis, add *CarolinaBLU*™ Gel and Buffer Stain in the amounts indicated in the table below. Note that the amount of stain added is dependent upon the voltage used for electrophoresis. *Do not use more stain than recommended. This may precipitate the DNA in the wells and create artifact bands.*

Gels containing *CarolinaBLU*™ may be prepared one day ahead of the lab day, if necessary. However, gels stored longer tend to fade and lose their ability to stain DNA bands during electrophoresis.

Use the table below to add the appropriate volume of *CarolinaBLU*™ stain to the agarose gel:

Voltage	Agarose Volume	Stain Volume
<50 Volts	30 mL	40 µL (1 drop)
	200 mL	240 µL (6 drops)
	400 mL	520 µL (13 drops)
>50 Volts	50 mL	80 µL (2 drops)
	300 mL	480 µL (12 drops)
	400 mL	640 µL (16 drops)

Use the table below to add the appropriate volume of *CarolinaBLU*™ stain to 1× TBE buffer:

Voltage	Agarose Volume	Stain Volume
<50 Volts	500 mL	480 µL (12 drops)
	3000 mL	3 mL (72 drops)
>50 Volts	500 mL	960 µL (24 drops)
	2600 mL	5 mL (125 drops)



## BIOINFORMATICS

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Have students do the bioinformatics exercises before starting the experiment—or analyzing results. This should improve conceptual and practical understanding.

The onscreen Bio-i Guide can be played from the included CD-ROM or from the Internet site <http://bioinformatics.dnalc.org/pv92/>. The default version (640 x 480 pixels) allows one to follow along with an open browser window. The full screen version (1024 x 768 pixels) is best for demonstrations.

## ANSWERS TO BIOINFORMATICS QUESTIONS

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- I.2. a. Matches of different lengths are coded by color. What do you notice? **There is only one complete match to the forward and reverse primers, followed by a number of partial matches.**
- I.3. b. Note the names of any *significant alignments* that have *E-values* less than 0.1. Do they make sense? **There is only one hit with an *E-value* of less than 0.1. It makes sense, because it is from human Chromosome 16.**
- I.3. d. The lowest and highest nucleotide positions in the subject sequence indicate the borders of the amplified sequence. Subtracting one from the other gives the difference between the two coordinates. **57137 – 56722 = 415.**
- I.3. e. However, the actual length of the fragment includes both ends, so add 1 nucleotide to the result to determine the exact length of the PCR product amplified by the two primers. **416 nucleotides.**
- I.3. f. Is this the + or the – allele? **There is not enough information to tell yet.**
- II.4. What do you notice about the *E-values* obtained by this search? Why is this so? **Three hits have extremely low *E-values* (have many decimal places). This is because the query sequence is longer.**
- II.5. Why does the first hit have an *E-value* of 0? **This hit completely matches the query, because it is the same Chromosome 16 clone identified in Part I.**
- II.6. a. What do you notice about the 3' end of the *Alu* repeat? **There is a poly-A tail composed of a string of 28 As (adenines).**
- II.6. b. What appears to be going on? **The target sequence gaaagaa is duplicated during the insertion of the *Alu* element.**
- II.7. What is the length of the *Alu* inserted at PV92? **The PV92 *Alu* is 308 bp long.**
- II.8. If you assume that the amplicon in Part I is the – allele, what is the length of the + allele? **The + allele would appear to be the sum of 416 bp + 308 bp = 724 bp. However, the + allele also includes the 7-bp duplication of the target sequence. So the actual length of the + allele is 731 bp.**
- II.9. Now look carefully at the third low *E-value* hit. Examine the *Features* and follow links. What is going on here? How are the three hits related to one another? **There are annotations for *Alus* belonging to two different subfamilies: Ya5 is the older group that includes PV92, and Yb8 is a younger group. The younger *Alu* jumped inside the original *Alu* at the PV92 locus. One can easily see two poly-A tails in the sequence – one belonging to each *Alu*. This *Alu* within an *Alu* allele is rare and inserted so recently that it has only been found in a few people, notably from the Basque region of Spain and northern Morocco.**
- III.4. On what chromosome have you landed? **Chromosome 16.**
- III.7. What can you say about the gene that contains the amplicon? Click on the name in the *Genes\_seq* track, then follow links to find out. **The amplicon lies within the cadherin H 13 (CDH13) gene. This gene produces a cell adhesion protein that mediates interactions between cells in the heart.**

- III.8.a. Determine the size of the CDH13 gene using the map coordinates to the left of the contig map. **CDH13 is approximately 1.2 million nucleotides in length.**
- III.8.b. How many introns and exons does CDH13 gene have? **CDH13 has 13 exons and 12 introns.**
- III.8.c. Where in the CDH13 gene is PV92 *Alu* inserted: an exon or intron? **PV92 *Alu* is inserted within the 2<sup>nd</sup> intron.**
- III.8.d. How does this explain the fact that the PV92 insertion is believed to be neutral—having no phenotypic effect? **Mutations within introns generally have no phenotypic effect.**

## ANSWERS TO DISCUSSION QUESTIONS

*Instructions on how to set up your class data are on the website and the CD-ROM.*

2. An *Alu* insertion has only two states: + and -. How does this relate to information stored in digital form by a computer? What equivalent in 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.**
4. Is the + allele confined to any particular racial or ethnic group? What can you say about people in the class who have at least one + allele? **The + allele is not exclusive to any racial or ethnic group. All people who have at least one + allele inherited their allele(s) from a common ancestor.**
5. b. How do genotype frequencies you observed in your experiment compare with those expected by the Hardy-Weinberg equation? Would you say they are very similar or very different? **Observed genotype frequencies typically are quite similar to the expected frequencies.**
7. h. Is your p-value greater or less than the 0.05 cut-off? What does this mean? **Class results typically have p-values greater than 0.05. This means that there is not a significant difference between observed and expected frequencies and that the observed frequencies are consistent with Hardy-Weinberg equilibrium.**
7. i. What conditions are required for a population to come into genetic equilibrium? Does your class satisfy these requirements? **Genetic equilibrium requires a relatively large population, no migration in or out of the group, no new mutations at the locus under study, and random mating in relation to the locus. While the class itself would be a very small population, its members are more or less representative of a larger population in your town or region. There is probably a relatively small amount of migration in and out of your town or region. There is no evidence of very recent, new mutations at the PV92 locus that would influence genotypes. There is no way of telling a person's PV92 genotype by looking at them, so people mate randomly in relation to this polymorphism. So, perhaps surprisingly, the class may generally fulfill the requirements for Hardy-Weinberg equilibrium.**
8. i. Which groups have significantly different genotype frequencies? What is the most frequent genotype in each group? **European, African, Australian, and American populations typically have similar genotype frequencies, with the -/- genotype being most common. The +/+ genotype is most common in Asian populations.**
9. g. Do you notice any pattern in the allele frequencies? **The + allele frequency is high in all Asian groups (up to 90%) and generally decreases moving westward through the Middle East, with European and African populations having frequencies of 10-35%. High + allele frequencies are also found in American Indian populations: Yanamamo (96%) and Maya (70%).**

9. h. Suggest a hypothesis about the origin and dispersal of the *Alu* allele that accounts for your observation. **Most students conclude that this pattern is consistent with the PV92 *Alu* insertion arising in Asia and then being diluted by gene flow to the west. Well-studied students, especially after doing exercise 10 (below), may understand that the pattern could also be the product of migration and genetic drift.**
9. i. Calculations suggest that the PV92 insertion occurred about 200,000 years ago. If this is so, in what sort of hominid did the jump occur, and what implications does this have for your hypothesis from h. above? **The PV92 insertion would have occurred in a population of *Homo erectus*, which then survived to give rise to modern humans (us). If this jump occurred in Asia, then *Homo erectus* must have survived in Asia to give rise to modern populations there. This would be consistent with the regional development hypothesis. The accepted replacement hypothesis—also called “Out of Africa”—supports the PV92 insertion occurring in a *Homo erectus* population in Africa. The worldwide frequencies of approximately 20% suggest that the + allele drifted to approximately this frequency in Africa prior to the migrations that gave rise to European, Asian, and Australian populations. The frequency then drifted much higher among the migrants that founded Asian populations, several of which may have carried a high + allele frequency when they migrated across the Bering Strait to found American Indian populations.**
10. d. How did hominids live 200,000 years ago, and what size population group would be supported? **Our hominid ancestors existed only by hunting and gathering, so that would limit the size of each group to around 50 individuals.**
10. e. What would be the allele frequency if a new *Alu* jump occurred in a group of this size? **For example, 50 people in the hunter-gatherer group would have 100 alleles, with one having the new *Alu* insertion – for an allele frequency of 1%.**
10. m. What happens to the new *Alu* insertion in the 100 populations? **The + allele frequency decreases from 1 percent to 0 percent in most of the populations within about 10 generations. The new *Alu* mutation is lost from these populations. Typically, the + allele is maintained in several populations at the end of 100 generations. Occasionally, the + allele will be fixed in a population, when the frequency rises to 100%.**
10. n. Follow the allele frequency in one population over 100 generations. What happens to the allele frequency, and what causes this? **The + allele frequency changes dramatically within one population. This random fluctuation in allele frequency is termed genetic drift.**
11. i. What do you notice about the allele frequency in those populations that maintain the + allele over 200 generations? **The + allele frequency drifts during the first 100 generations, but stabilizes in the expanded population. The larger population is nearing Hardy-Weinberg equilibrium.**

**PV92 “+” Allele Frequencies**

Kung	20
African American	20
Alaska Native	29
Australia Aborigine	15
Breton	27
Cajun	21
Chinese	86
Euro-American	18
Filipino	80
French	23
German	10
Greek, Cyprus	18
Hispanic American	51
Hungarian	12
India Christian	48
India Hindu	52
Indian Muslim	30
Italian	24
Java	84
Malay	72
Maya	70
Moluccas	69
Mvsoke	53
Nguni	24
Nigerian	9
Pakistani	30
Papua New Guinea	14
Papua New Guinea (Coastal)	19
Pushtoon	33
Pygmy (Central Africa)	26
Pygmy (Zaire, now Congo)	35
Sardinian (Aritzo)	17
Sardinian (Marrubiu)	00
Sardinian (Ollolai)	00
Sardinian (San Teodoro)	27
Sotho	29
South India	56
Swiss	20
Syrian	18
Taiwanese	90
Turkish, Cyprus	58
United Arab Emirates	30
Yanomamo	96

## CD-ROM CONTENTS

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The valuable companion CD-ROM is for exclusive use of purchasers of this DNA Learning Center Kit. To accommodate home or computer lab use by students, all materials may also be reached at the companion Internet site <http://bioinformatics.dnalc.org/pv92/>.

- **Protocol:** a unique online lab notebook with the complete experiment, as well as printable PDF files.
- **Resources:** 13 animations on key techniques of molecular genetics and genomic biology, from the award-winning Internet site, *DNA Interactive*.

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