The Science and Issues of Human DNA Polymorphisms:
An ELSI Training Program for High School Bioloogy Teachers
DNA Learning Center (DNALC), Cold Spring Harbor Laboratory

Objectives

The Science & Issues of Human DNA Polymorphisms Workshop trained high school science teachers to implement an educational analog of the Human Genome Project that "personalizes" gene technology and provides unique learning opportunities for high school students:

- To participate in an evolving project on human variability and population genetics.
- To collect, analyze, and share data on human DNA polymorphisms.
- To gain insight into the possibilities and limitations of DNA typing/databasing.
- To use online data storage and statistical facilities at the *Bioservers* WWW site (http://www.bioservers.org/bioserver/).

Significance and Relationship to DOE Genome Program

It will be tempting for students (and the general public) to view the Human Genome Project's catalogue of normal and mutated human genes as some authoritative measure of human life – and as a rational basis for further differentiating people by race, class, or ethnic origin. Teachers need to counter such simplistic notions of "good gene/bad gene" with a broader view obtained from the study of human evolution – that human beings are more alike genetically than they are different. Eminent human geneticist Luca Cavalli-Sforza has said that understanding our common genetic ancestry – and the essential likeness – of all human beings may be the best inoculation against racism for young people growing up in a world that increasingly emphasizes cultural, racial, and ethnic differences.[2]

How, for example, can teachers help students come to the subtle understanding that genetic testing entails value judgements about what is normal *versus* abnormal – and that the social and legal acceptance of such judgements can create a pressure for genetic conformity? Most high school teachers attempt to stimulate this type of higher-order social and ethical analysis through case studies, role-playing, and panel discussions. Each of these methods require students to identify situations normally outside their own experience. Furthermore, students' opinions can be substantially colored by the teacher's presentation or selection of the necessary "facts" on which the exercise is based.

In contrast, the DOE Training Program offered the novel alternative of a laboratory-based unit on DNA polymorphisms that encourages students to use their own experience with current genetic technology as a basis for evaluating the ethical, legal, and social aspects of science. Students amplify their own DNA polymorphisms and use computer analysis tools to discover how humans are related at the genetic level. In combination with the program's forward-looking resources that enable students to feel a part of genomic biology, we also provide the opportunity for them to look back to a previous era of involvement in human genetics.

We believe that this is a powerful system for involving students in the social analysis of genome research. Students are challenged to expand from the personal view of their own DNA polymorphisms to consider the shared genetic ancestry of all human beings. This is in line with the concept of "anchored instruction," where students use lab and computer learning tools to help them pose and solve meaningful problems.[3] A student's questions about his or her own DNA polymorphisms engender much of the ethical and personal decision making of human genetics:

- Are my polymorphisms rare or frequent (in my class, in the world)?
- Do I share polymorphisms with people of other races and ethnic groups?
- How genetically alike or different are people (of different countries, different races)?
- Would I want to know the results of my polymorphism analysis if it was a diagnosis of susceptibility to a serious or life-shortening disease? (The *Alu* insertion and mitochondrial control region polymorphisms used in the program are, in fact, phenotypically neutral – having no relationship with any human characteristic or disease.)
- Would I want *others* to know about my polymorphism analysis if it was a disease diagnosis?

Relation to Present State of Knowledge

During the last 20 years, the molecular genetic perspective has helped to integrate the many subdisciplines of biology. Key to this has been the power of recombinant-DNA techniques to dissect the genomes of many organisms and to emphasize the continuity of life at the genetic level. The Human Genome Project represents the culmination of this approach and its systematic application to the problems of human health. There is increasing awareness among educators that the molecular genetic perspective can also unify biology education. According to the National Science Education Standards, molecular and evolutionary biology are among the "small number of general principles that can serve as the basis for [high school] teachers and students to develop further understanding of biology."[4]

Modern genetics offers an almost unparalleled opportunity to integrate concepts from biology, chemistry, and physics – as well as the personal and social implications of new technologies. This is consistent with whole learning, across curricula, and science-technology-society (STS) approaches that synthesize information from various disciplines and relate learning to the student's personal life and culture.[5] Systematic genetics education during high school would fulfill three important objectives, which essentially parallel student development:

- To inculcate basic tenets of genetic literacy that are essential for all students as they assume management of personal and family health care as adults.
- To help prepare college-bound students for their future roles as opinion leaders in government, industry, education, medicine, and law.
- To maintain and broaden the involvement of science-interested students who are intent on biology or health-related majors, and to stimulate this interest in other motivated students.[6]

The National Science Education Standards also stress that, "In grades 9-12, students' understanding of biology will expand by incorporating more abstract knowledge, such as the structure and function of DNA..."[4] However, molecular genetics is almost maliciously abstract for even bright students. The DNALC and other proponents of laboratory instruction in molecular genetics argue that doing experiments is probably the only way to reduce this abstraction. The Educational Testing Service legitimized the importance of laboratory experience in molecular genetics as a basis for student understanding when, in 1989, it recommended two DNA-manipulation labs for students who take its nationally-administered *Advanced Placement (AP)* Biology curriculum: DNA restriction analysis and DNA transformation.[7] This was followed by the inclusion of these same labs in *Applications in Biology/Chemistry*,[8] the national Tech Prep curriculum that is in line with the National Science Education Standards for science and technology education.

While this represented a great step forward in technical education, in fact, this has only brought instruction to the state-of-the-art of 20 years ago. These experiments illustrate several generic techniques of biotechnology, but do not illustrate the intellectual synthesis offered by genomic biology – nor do they provide a focus on human biology and its problems. Thus, there is still much to do to help bring biology

instruction into the era of the human genome.

The AP and Tech Prep mandates catalyzed the rapid adoption of several core techniques of molecular genetic manipulation. According to a survey we conducted with National Science Foundation (NSF) funding, about 8,000 high school faculty currently teach hands-on labs on DNA manipulation, and about 1,500 offer biotechnology/molecular genetics electives.[1] The DOE Training Program targeted this audience of highly professional faculty – who also attend professional meetings, develop curricula, collaborate with scientists, teach faculty workshops, and manage equipment-sharing programs. Providing follow-up training to these lead teachers, who have proven networking abilities, is potentially a cost-effective means to disseminate new methods for involving students in the Human Genome Project.

However, this comes at a time when the NSF has essentially abandoned its support of individual leaders in favor of system-wide initiatives. The DNALC is probably the only academic institution in the U.S. that has the capability to routinely administer high-level training in molecular genetics at sites around the country. The DNALC's reputation for innovative instruction, the timeliness of the subject, plus the 2.5-day format has translated into a strong demand for the DOE Workshop. Although we accepted 7% more qualified participants than budgeted in the proposal, the 256 participants in the current workshop series represent only a small fraction of teachers who could benefit from the experience. Thus, there is continued need for DOE's sponsorship of *The Science & Issues of Human DNA Polymorphisms*.

Using student polymorphisms and online statistical tools to explore human relatedness is in line with several of the content standards for grades 9-12 of the National Science Education Standards.[9] Content Standard A, "Science as Inquiry," stresses that students should participate in meaningful investigations that encourage them to ask questions, use technology and mathematics, and formulate and revise models. Our formatted exercises help students, for example, see how different interpretations of their own *Alu* and mt sequence polymorphism data may support alternative models of human evolution.

Content Standard C, "Life Science," emphasizes the molecular basis of heredity and biological evolution as key to deepening student understanding of biology. However, molecular genetics is also singled out as a perfect example of Content Standard F, "Science in Personal and Social Perspectives." "Because molecular biology will continue into the 21st century as a major frontier of science, students should understand the chemical basis of life, not only for its own sake, but because of the need to take informed positions on some of the practical and ethical implications of humankind's capacity to tinker with the fundamental nature of life."[1]

The extent to which race is a biological *versus* a social construct can frame an important discussion on how cultural and personal beliefs may influence science. In conducting their own analysis of population genetic data, students can internalize the modern notion that living humans share a recent evolutionary history and are remarkably similar to one another. Then they can see that this is at odds with any preconceived notion that some population groups are inferior to others.

Relation to Long-term Goals and Other Work in Progress

The social imperative of genetics research demands the development of educational resources to build a genetically literate public that supports basic biological research, understands elements of personal genetic health, and participates effectively in policy issues involving genetic technology and information. The magnitude of this task argues for the creation of regional "Human Genome Education Centers," which provide stable, well-equipped environments in which multi-disciplinary teams of biologists, educators, designers, and computer programmers could devote their creative energies entirely to the problems of public genetics education. This approach acknowledges that innovation arises from a coordinated, critical mass of highly motivated individuals and directly parallels well-established research

models – such as the National Institutes of Health (NIH) and Department of Energy (DOE) Human Genome Research Centers, and the NIH Cancer Centers and Program Project Grants. Our long-term goal is to continue the development of the DNALC as a prototype of such a genome education center – with a comprehensive public education program that merges the theory, practice, and social implications of human gene manipulation.

The ELSI Training Program was the culmination of the DNALC's systematic program to remove the obstacles to using human polymorphisms in education by simplifying the biochemistry for amplifying human DNA polymorphisms and by providing simple bioinformatics tools for analyzing student results. DNALC staff began experimenting with the human VNTR polymorphisms in 1989 – developing the first educational D1S80 kit in collaboration with Perkin-Elmer scientists as they developed the first forensic D1S80 kit. DNALC teaching kits were among the first in the country to optimize the use of Chelex with DNA preparation by saline mouthwash – a method we still find produces the best results in student hands. Beginning in 1992, DNALC staff worked with world authorities Prescott Deininger and Mark Batzer, of LSU Medical Center, to develop the *Alu* insertion polymorphisms for educational use. Collaboration with the bioscience computing group at the University of Chicago, beginning in 1994, led to the development of a prototype Internet-accessible facility to store and manipulate polymorphism data (*Student Allele Database*) and of an inexpensive computer-controlled thermal cycler (*Biogenerator).* In 1997, we received an educational license to market the *Biogenerator,* a computer-driven thermal cycler based loosely on Bob Watson's "PCR in a Teacup."[10]

In 1998, Carolina Biological Supply Company released three *Advanced Technology (AT)* human PCR kits, developed by the DNALC, which make use of a robust three-part chemistry:

- *Human* Alu *Insertion Polymorphism Kit AT* assays for the presence (+) or absence (-) of the *Alu* transposable element at the PV92 locus on chromosome 16 (Catalog # 21-1232).
- *Human VNTR Polymorphism Kit AT* assays for a VNTR (variable number of tandem repeats) polymorphism at the pMCT118 locus on chromosome 1 (Catalog # 21-1235).
- *Human Mitochondrial DNA Kit AT* amplifies a 460-nucleotide sequence within the control region of the mitochondrial genome, which can then be sequenced to show single nucleotide polymorphisms, or SNPs (Catalog # 21-1238).

ELSI Workshop Program

Over the term of the grant, we conducted 12 workshops were attended by 256 high school faculty, exceeding proposed attendance of 240 by 7%. We collaborated with higher education institutions to conduct workshops in 11 different states:

October 24-26, 1997	Mt. Sinai School of Medicine	New York, New York
November 8-10, 1997	Boston University School of Medicine	Boston, Massachusetts
November 14-16, 1997	Cañada College	Redwood City, California
December 5-7, 1997	Morehouse College	Atlanta, Georgia
March 26-28, 1998	Eccles Institute, University of Utah	Salt Lake City, Utah
November 12-14, 1998	Mills Godwin Specialty Center	Richmond, Virginia
December 3-5, 1998	University of Denver	Denver, Colorado
January 9-11, 1999	California Lutheran University	Thousand Oaks, California
March 26-28, 1999	Fred Hutchinson Cancer Center	Seattle, Washington
December 3-5, 1999	Laney College	Oakland, California
April 3-5, 2000	University of Miami School of Medicine	Miami, Florida
April 7-9, 2000	Austin Community College	Austin, Texas

Project staff collaborated with local organizers to distribute workshop announcements and recruit participants. In our experience, adequate numbers of qualified applicants are best recruited by direct mail to individual teachers, rather than to administrators or department chairs. Based on a 3% response, we

mailed approximately 750 applications per site. The local organizers secured appropriate regional lists available from state departments of education and state science teachers' associations. In addition, we purchased mailing labels for AP and Tech Prep teachers in the target region from Market Data Retrieval. Schedules and materials were also distributed at the NABT and NSTA national conventions, and posted on computer bulletin boards at the DNALC WWW site and the host institutions. Candidates completed a four page application, which provides data on seven major selection criteria:

- Academic preparation – evidence of advanced degree or coursework of special relevance.
- Appropriate teaching assignments – AP Biology, Tech Prep Biology, Biology/Chemistry, Honors Biology, Biology II, or advanced elective.
- Professionalism – involvement in curriculum development, departmental affairs, and professional societies.
- Student enrichment – involvement in student research, science fairs, and/or field trips.
- *Applicant minority status.*
- *School percentage of minority/disadvantaged students.*
- Institutional support – signature of support statement by principal and/or department chairperson; voluntary letter of recommendation.

The ELSI Training Program was modeled after the Cold Spring Harbor Laboratory Postgraduate Training Courses, which are characterized by their intensity. The ELSI Training Program included several elements:

- *Biochemistry Laboratories* illustrated the experimental analysis of human DNA polymorphisms and methods of inference about gene structure and function. Participants learned all steps of the analysis: preparing DNA extracts, setting up PCR reactions, separating alleles by gel electrophoresis, staining gels, and photographing results. Pre-lab sessions focused on proper technique and alerted participants to critical points in the procedure. Post-lab sessions provided an opportunity to interpret and share results – observing the range of anomalies helps participants learn to troubleshoot reagent and procedure variables. Practical advice was given on laboratory management techniques, including preparation, scheduling, alternative methods/equipment, and procedure break points. (The polymorphic systems used in the program are described in greater detail below.)

- *Computer Laboratories* made use of a suite of user-friendly software for statistical analysis of polymorphism data generated by the lab experiments. (Computer tools used in the program are described in greater detail below.)

- *Concept Seminars* introduced key theoretical background to the biochemistry and computer labs – including the biology of human polymorphisms, PCR biochemistry, shuman population biology, and pharmacogenetics. To insure continuity, concept seminars were presented by Project Staff.

- *Feature Seminars* were presented by local scientists or ELSI experts to provide first-hand insight into the uses and issues of human DNA polymorphisms in human genome research. Seminar topics varied from site to site, making use of the best available local speakers. Topics at past workshops have included gene mapping and cloning, DNA diagnosis, population genetic screening, DNA databanking, gene mining in populations, genetic privacy, and insurance issues.

- *Discussions* encouraged participants to brainstorm for ideas about how they can implement experiments and ethical debates in their schools; how to link the DOE program to state and national science standards; how to use their supply stipend; and how to share the PCR footlocker.

Follows is a representative workshop agenda used at the Mailman Center of the University of Miami:

Monday, April 3

8:30 AM	Registration/Coffee	
9:00 AM	Welcome/Introductions	
9:30 AM	Concept Seminar	Genomes and DNA Polymorphisms
11:00 AM	Concept Seminar	Theory and Applications of PCR
12:00 PM	Lunch	
1:00 PM	Biochemistry Lab	Isolate DNA from Mouthwash and Hair Follicles Construct Biogenerator Set up PCR Reactions for *Alu* insertion, VNTR, and mDNA Amplify using *Biogenerator* and Commercial Thermal Cycler Prepare Agarose Gels
4:00 PM	Seminar	*Microarrays and Pharmacogenomics* Karl Muench, University of Miami School of Medicine
5:30 PM	Adjourn	

Tuesday, April 4

8:30 AM	Coffee	
9:00 AM	Biochemistry Lab	Load and Electrophorese Amplified Samples
12:00 PM	Lunch	
1:00 PM	Biochemistry Lab	View and Photograph Gels
2:00 PM	Concept Seminar	VNTRs, the *Alu* Lifestyle and Identity by Descent
3:00 PM	Computer Lab	Population Genetics: *Student Allele Database* Enter Class Data Hardy-Weinberg Equilibrium and Population Comparisons
4:00 PM	Seminar	*Bioinformatics and the E. coli Genome Project* Ken Rudd, University of Miami School of Medicine
5:30 PM	Adjourn	

Wednesday, April 5

8:30 AM	Coffee	
9:00 AM	Seminar	*Genetics Investigatrion in a Bahamian Population: Contemparory and Historical Perspectives* Lisa Baumbach, University of Miami School of Medicine
10:30 AM	Concept Seminar	The Mitochondrial Clock and Human Evolution Mitochondrial DNA Reconstructing Neandertal and Romanov Sequence Analyses
11:4 5 AM	Discussion	Workshop Summary Implementation and Supply/Equipment Stipend
12:30 PM	Adjourn	

Teacher and Instructional Effects

In February-March 2000 and 2001, we conducted a follow-up mail survey of 256 faculty who participated in workshops conducted during the current term of DOE support. Seventy percent of participants responded, providing direct reports on how their teaching behavior had changed since taking the DOE workshop. About nine of ten respondents said they had provided new classroom materials and first-hand accounts of DNA typing, sequencing, or PCR. Three-fourths had introduced new units on human molecular genetics. Most strikingly, half had students use PCR to amplify their own insertion polymorphisms (PV92), and better than one-fourth amplified a VNTR polymorphism and the mitochondrial control region. One in five had mitochondrial DNA sequenced by the DNALC *Sequencing Service*. A majority (58%) used online materials at the DNALC WWW site, and 28% analyzed student polymorphism data with *Bioservers* at the DNALC site. A majority (58%) assisted other faculty with student labs on polymorphisms, reaching an additional 786 teachers.

A previous study we did of 4,000 innovative biology teachers strongly suggested that non-respondents had similar levels of behavior change as respondents[1]. Considering that the professional and teaching attributes of faculty trained at DOE workshops closely match those in the larger study, we feel justified in extrapolating behavior rates for respondents to the entire cohort of teachers trained under DOE grant. The following table provides percent of teacher behaviors (%), reported number of students and faculty exposed (N Exp), and extrapolated number of students and faculty exposed (E Exp):

Behavior (190 respondents)	**%**	**N Exp**	**E Exp**
Gave first hand account of DNA typing, sequencing, or PCR	92	20,561	27,703
Provided new material on DNA typing, sequencing, or PCR	92	20,639	27,808
Implemented new unit on human molecular genetics	74	16,994	22,897
Had students amplify PV92 or other insertion polymorphism	52	7,342	9,892
Had students amplify D1S80 or other VNTR polymorphism	28	2,417	3,257
Had students amplify mitochondrial control region	29	2,713	3,655
Had mitochondrial control region sequenced at DNALC	21	1,844	2,485
Used online materials at DNALC WWW site	58	10,578	14,242
Had students analyze their own DNA polymorphisms online	28	2,948	3,972
Helped other faculty do a human PCR experiment	58	786	1,059

Experiments from the DOE-sponsored program are being replicated on a large scale at locations around the United States. Notably, the Bay Area Biotechnology Education Consortium (BABEC), in San Francisco, has adopted the PV92 *Alu* lab for its kit program – reaching 3,700 students per year. The BABEC program is sponsored by Applied Biosystems, which also has adopted our mt DNA sequencing lab for field trips to its facility in Foster City. Sponsored by the Howard Hughes Medical Institute, 800 students performed the human polymorphism labs in Montgomery County, Maryland. A teaching lab and DNA sequencer are integral parts of the exhibit, "The Genomic Revolution," which opens in May 2001 at the Museum of Natural History. Both human labs will be done by students visiting the exhibit, as well as those using mobile kits circulated to New York City Public Schools.

The DOE-sponsored program received wide recognition at the *Decade of ELSI Research* meeting held at the National Institutes of Health (NIH) on January 16-18, 2001. It was the only precollege education program selected for plenary presentation at this meeting, which drew more than 400 participants.

Laboratory Methods Used in the Program

In developing the laboratory sequence, we have attempted to address practical, safety, and ethical concerns that might limit classroom use.

- Sample preparation from cheek cells is noninvasive and bloodless.
- DNA extraction using Chelex involves no toxic or organic solvents.
- Ready-to-Go Beads are extremely stable and improve classroom reproducibilty.
- Classroom analysis of PV92 alleles and of the mt control region amplicon require only agarose mini-gel systems typically used in educational settings.
- Amplification of both PV92 and the mt control region are extremely robust, yielding sufficient products for staining with methylene blue or other nonmutagenic stains.

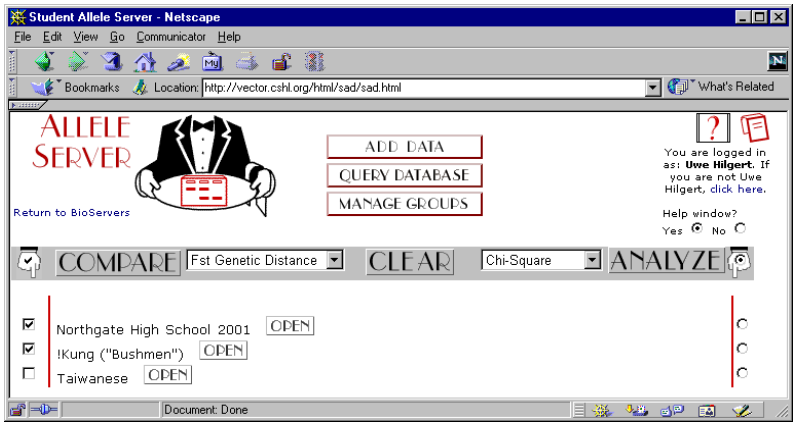
We considerably extended the scope of the training program proposed in the grant application when, in mid-1997, we introduced reproducible technology for generating DNA sequence of the mitochondrial (mt) control region. During each DOE workshop, teacher participants amplified the mt control region from DNA prepared from their hair roots or cheek cells. The mt DNA amplicons were returned to the DNA Learning Center (DNALC), where they were prepared for cycle sequencing at the Cold Spring Harbor Laboratory (CSHL) Genome Sequencing Center. The completed sequences were then posted at the DNALC WWW site for analysis.

In addition to processing sequences from teacher workshops, we also processed 1,800 student mt DNA samples submitted by teachers we trained around the country. All submitted mt DNA amplicons were processed by a high school intern, who performed dye terminator reactions and DNA precipitations and then passed the samples on to the Sequencing Center. Using a web-based sequence manager, DNALC staff then tracked the submitted samples, queried the Genome Center server, returned the completed sequence into the DNALC database, and e-mailed results to the teacher. Teacher and student mt DNA sequences were made available for analysis at the *DNA Sequence Server* site (http://vector.cshl.org/html/sequences/sequences.html). Thus, we provided proof of concept for a *Sequencing Service* to process mt DNA samples submitted from biology classes around the country.

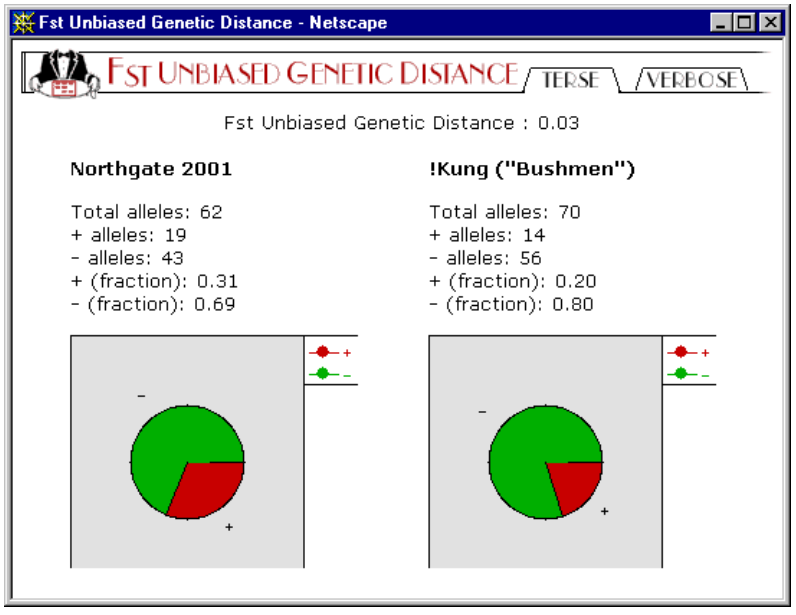
Computer Tools Used in the Program

Early in 2001, we developed a meta-website, *Genetic Origins* (http://vector.cshl.org/geneticorigins/), which draws togther all of the computer resources to support the ELSI Training Program. This site includes downloadable laboratory protocols, reagent recipes and supporting animations, as well as video interviews with molecular anthropologists and custom bioinformatics tools:

- *Allele Server* allows students to tabulate student *Alu* insertion data and compare two populations by contingency chi-square, genetic drift, and genetic distance. The database has forms to allow students to enter their own genotypes into the database, and reference data from more than 40 world populations (provided by Mark Batzer, of LSU Medical Center and Marcello Siniscalco, of the Coriel Institute). Examining PV92 in these world populations shows a distinct East-West cline, with the insertion allele nearly fixed in Southeast Asian populations and diminishing through India, Europe, and Africa. This leads students to propose two alternative mechanisms to account for this observation: 1) The insertion arose in Southeast Asia and spread westward by migration and gene flow. 2) The insertion arose in Africa and drifted to higher frequency in a founding population of Southeast Asia.



- *Simulation Server* allows students to model genetic changes over time – simulating the same conditions in 100 or more test populations at a time. Teachers appreciate this facility, because it allows students to model Hardy-Weinberg equilibrium in model gene systems under selective pressure – such as the sickle cell mutation. However, in the context of PV92, the facility can help students understand non-equilibirum circumstances under which neutral alleles drift toward extinction or fixation. For example, students are encouraged to envision a time when proto-humans or early modern humans



lived in small hunter-gatherer groups. What happens to a new *Alu* insertion that occurs in such a small group, and after 1,000 generations when the group adopts agriculture and expands in size?

- *Sequence Server* allows students to enter their own mt sequence data and perform multiple sequence alignments. As with the *Alu* data, they may compare themselves to other students or to world reference populations and ancient DNA samples. They may also align their sequence against the entire mt genome to determine the location of the control region. A student may also use his/her mt DNA sequence to perform a BLAST search to find similar sequences in the primary gene repository, *Genbank.* Sequences identified in the search can then be moved to the student's workspace for further comparison.