A DNA Science research and training programme for Secondary School and Junior College teachers and students on genetic polymorphisms in human, animals and plants in Singapore

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Abstract

The objective of this project is to train teachers and students to be competent in the principles and practice of DNA science by working on genetic polymorphisms of humans, animals and plants in Singapore. MOE has provided JC and Secondary Schools in Singapore the life sciences research facilities and equipment which cost millions of dollars. This project is therefore timely and useful to support the life science initiative of MOE in education.

This project has both educational and research benefits. The data collected are important from the scientific point of view, as well as for educational purpose for schools in Singapore. Teachers and students, through this project, will be exposed to the exciting and innovative world of DNA Science, including forensic and biomedical DNA, so that they may appreciate the myriad career opportunities available to people with good knowledge in life sciences and biomedicine. Nine schools have indicated their interests in joining this project as pilot schools.

Data collected from the study of human genetic polymorphisms will form the DNA database of human variation for the different ethnic populations. This is valuable information for forensic DNA science in Singapore. The human genetic polymorphisms of various DNA markers; plant and animal polymorphisms will be studied. Teachers and students will be trained the knowledge of bioinformatics and how to access and assess DNA database obtained from the Human Genome Project and the method of how to test GMO food. A national DNA Science Symposium for Singapore schools will be organized at the end of the period for students from participating schools to present their findings.

Introduction

All Junior Colleges (JC) and Secondary Schools in Singapore have been encouraged by the government to nurture students to learn new knowledge in life sciences and biomedicine. It is important that talented scientists and technicians are required to apply the principles of molecular biology and genetics to research and innovations in the biomedical, pharmaceutical, veterinary, and environmental industries, which are the engines that drive the economic growth of Singapore in the third millennium. With this initiative, all JC and Secondary Schools have been given budget to develop the life science teaching and research facilities. Each school has been given various instruments that include a thermal cycler for the polymerase chain reaction (PCR), agarose gel electrophoresis equipment, micropipettes, microcentrifuge, autoclave, *etc*. These expensive instruments are worth hundreds of thousands of dollars. In order to fully use the facilities and equipment, the teachers and students in JC and Secondary School need to have guidance, knowledge, ideas and scientific skills in conducting teaching and research activities that use PCR and related facilities. Many JC and Secondary School teachers do not know or are not sure how to order oligonucleotide primers for use in PCR, which primers to use, and what kind of teaching and research activities can be conducted in schools.

Educational purposes of this programme:

1. To provide training and enrichment courses/workshops in the fields of life sciences and biomedicine to Secondary School and JC teachers in Singapore.
2. To prepare and nurture Secondary School and JC students to gain and apply their knowledge and skills in life science research in the emerging fields of biotechnology, genetics, microbiology and biochemistry.
3. To improve and enrich the research skills of both teachers and students in Singapore Secondary Schools and JCs to undertake life science research projects, as well as to equip them with the latest and most up-to-date technology and skills.
4. To be proactive to the impending changes in biology syllabi at the GCE "O" and "A" levels. The revised biology/life science school curricula will give increased emphasis on molecular biology and genetics. Given the revised syllabi, both teachers and students need revised curriculum and instruction materials that satisfy the objectives and contents of the new syllabi.
5. To impart to Secondary School and JC teachers the skills and knowledge to supervise students of their own schools, so that school teachers can conduct research projects independently in the future.
6. To train teachers and school students how to search for scientific literature and other research resources including E-learning.
7. To train teachers and students to gain knowledge of bioinformatics and how to access and assess DNA database obtained from the Human Genome Project.
8. To train Secondary School and JC teachers and students to improve their analytical skill and ability and to learn how to write scientific reports.
9. To expose Secondary School and JC teachers and students to the exciting and innovative world of DNA Science, including forensic and biomedical DNA, so that they may appreciate the myriad career opportunities available to people with good knowledge in life sciences and biomedicine.

Scientific purposes of this programme:

1. Data collected from the study of human, animal, and plant genetic polymorphisms will contribute to the DNA databases of human, animal, and plant variations in Singapore. The data on human genetic polymorphisms in the different ethnic populations in Singapore will be valuable information for forensic DNA science and anthropological study in Singapore.
2. To study human genetic polymorphisms of various chromosomal and mitochrondrial (mt) DNA markers, e.g., VNTR apolipoprotein B 3' end polymorphism, *Alu* sequence PV92, D1S80 repeat polymorphism, various well-characterized and internationally accepted forensic short tandem repeat (STR) markers, various well-characterized and internationally accepted forensic Y-STR markers, and the three hypervariable sequences in the mt DNA D-loop or control region.
3. To investigate plant DNA markers in the study of genetic polymorphisms and molecular phylogenetics of tropical plants. Some plants are difficult to classify by using the traditional morphological characteristics.
4. To study genetic polymorphisms in animals, particularly pedigreed horses, dogs, and cats, by using standard STR markers. The results generated will contribute to the DNA databases of pedigreed horses, dogs, and cats.
6. To teach teachers and students how to identify genetically modified (GM) foods by using DNA-based molecular methods.
7. To expose teachers and students to the social, legal, and ethical issues of DNA Science.

Why this DNA science learning programme is interesting to teachers and students in schools ?

In this programe, we aim to train teachers and students to understand the power of DNA technology and to expose them to this exciting technology for the future of the nation. Teachers and students will learn that written in each person's DNA is a record of our shared ancestry and our species' struggle to populate the earth. The study of DNA mutations reveals that all humans alive today can be traced to a common ancestor living in Africa about 150,000 years ago and that our species came close to extinction at several points in the last 100,000 years. Thus, although people may look rather different on the outside, at the genetic level all humans are closely related.

Each person's unique disease susceptibilities and responses to drugs are due, in large part, to the

balance between our uniqueness as individuals and similarities we share with others in our historical population groups. Although pharmacogenomics ultimately promises to precisely tailor disease treatment to a person's unique genetic makeup, better treatments will first become available to population groups that share genetic affinities. The deployment of personalized medicine will bring DNA profiles into the doctor's office and may exaggerate the sense of difference between people. This, then, is the primary goal of this DNA Science research programme from NIE: To allow teachers and students to use their own DNA variations (polymorphisms) at both chromosomal and mt DNA markers as a means to explore our shared genetic heritage and their implications for society. Furthermore, both teachers and students will also examine DNA polymorphisms in non-humans by extracting and using plant and animal DNA. This will enhance their knowledge of DNA polymorphisms in humans, plants, and animals. They will also carry out specific DNA tests or assays to identify genetically modified foods, an excellent learning experience to appreciate the power of DNA technology.

Social, legal, and ethical issues will be considered before all teaching and research activities are carried out. If necessary, appropriate approvals from relevant committes, e.g., the Ethics Committee, will be obtained.

METHODS

Since all JC and most (if not all) Secondary Schools in Singapore have PCR machines, it is feasible for teachers to introduce appropriate teaching and research activities that analyze human, plant, and animal DNA. In collaboration with Dolan DNAC, we have removed the obstacles to using human DNA polymorphisms in education – by simplifying DNA isolation and PCR biochemistry and by providing simple bioinformatics tools for analyzing the results.

In this projects, teachers and students learn:

1. the DNA extraction methods from the tissues of human, plants and animals;
2. the principles and application of polymerase chain reaction (PCR).
3. agarose gel electrophoresis to analyse DNA fragments;
4. DNA sequencing for the mitochondrial DNA polymorphisms;
5. Bioinformatics and database analysis.

This study focuses initially on a number of informative DNA variations: an *Alu* insertion polymorphism on chromosome 16 (PV92); a highly polymorphic region of the *apoB* 3' end length repeat on chromosome 2; a D1S80 or pMCT118 repeat polymorphism in chromosome 1; single nucleotide polymorphisms (SNPs) in the control region of the mt DNA; 15 well-characterized and internationally accepted forensic STR loci (CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, vWA, FGA, TH01, TPOX, D2S1338, and D19S433 – the first 13 loci are FBI's CODIS core STR loci); and 17 well-characterized and internationally accepted forensic Y-STR loci [DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, Y GATA C4 (DYS635), and Y GATA H4].

PV92 is a simple genetic system that illustrates Mendelian inheritance at the molecular level, with two alleles and three genotypes. PV92 data are readily analyzed by using population statistics. The mt DNA control region is one of the simplest regions of human DNA to sequence. With a high mutation rate, the mt DNA control region is the "classical" system for studying human and primate evolution. The amplifications and analyses of forensic STR and Y-STR markers will illustrate the principle of multiplex PCR and the use of allelic ladders in human identification - from establishing DNA databases to determining parentage, tracing ancestry or genealogy, identifying remains, matching specimens, analyzing forensic casework, assisting in criminal investigations, and providing additional genomic information in anthropological research.

In the classroom, students use a simple, 30-minute procedure to isolate DNA from cheek cells. Then, they mix their DNA extracts with freeze-dried PCR reagents to amplify the polymorphic regions. *Alu* genotypes can be fully analyzed in class by electrophoresis on mini agarose gels. Then, class genotype data are entered into a database at the *Bioservers* Internet site of the Dolan DNAC. After electrophoresis has confirmed amplification of the mt DNA control region, student DNA samples are sent to the National Institute of Education's *Sequencing Service* for further processing. At the National Institute of Education, teachers or students will be trained to run the DNA sequencing machine. They

label the submitted samples with fluorescent dye terminators and a 440-nucleotide sequence of each student's DNA is determined by using an automated sequencer.

The finished student DNA sequences are uploaded into a database at the *Genetic Origins* Internet site, and the teacher is notified by email. For the determination of STR and Y-STR alleles at specific loci, student DNA samples are amplified by using the AmpFLSTR Identifiler PCR Amplification Kit (Applied Biosystems) and the AmpFLSTR Yfiler PCR Amplification Kit (Applied Biosystems) and the sizes of the multiplex PCR amplified products are determined by using an automated sequencer with fragment analysis capability (hardware and software), e.g., an ABI 3130 Genetic Analyzer.

BIOSERVERS DATABASES AND TOOLS

BioServers/DNALC was initiated by our collaborator, Dave Micklos, at the Dolan DNA Learning Center (DNALC) in 1998. This support allows us to develop a robust analog of genome research based on students' analysis of their own DNA, including 1) rapid protocols to amplify several types of human DNA polymorphisms, 2) a comprehensive Internet site, 3) a gratis service to sequence student DNA samples, and 4) training programmes to introduce the project to JC and Secondary School biology teachers.

Genetic origins(www.geneticorigins.org) and *BioServers* (www.bioservers.org)

Students access their *Alu* data or mt DNA sequences *via* the Internet and use statistical tools at the *BioServers* Internet site. *Sequence Server* and *Allele Server* are full-featured databases built on Microsoft SQL Server database technology. The applications let students access DNAC datasets, search external databases, and directly manipulate polymorphism collections. Students who register with the site can return to experiments saved in their workspace. *Simulation Server* is a "Monte Carlo" statistical programme that models population genetics.

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 the forms to allow students to enter their own genotypes into the database, and reference data from more than 40 world populations. 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-equilibrium circumstances under which neutral alleles drift toward extinction or fixation. For example, students are encouraged to envision a time when early humans lived in small hunter-gatherer groups. What happens to a new mutation 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 DNA 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. A student may also use his/her mt DNA sequence to perform a BLAST search to find similar sequences in GenBank. Sequences identified in the search can then be moved to the student's workspace for further comparison. Two-way and three-way comparisons between mt DNA samples from modern human populations can easily show the DNA support for a recent common ancestor in Africa: 1) African groups show the greatest number of mt mutations. 2) Most Asian, European, and New World mutations are a subset of African mutations. The addition of the three available Neandertal samples to this analysis can show this ancient hominid's relationship to modern humans.

In collaboration with DNA Learning Centre(DNALC) of the Cold Spring Harbor Laboratory (CSHL)

CSHL has played a major role in the development of modern genetics and has been home to four Nobel Prize winners – including the current Chancellor, Prof. James D. Watson. Watson and Crick are the scientists who received the Noble prize based on their fascinating discovery of DNA structure. DNALC has pioneered the development of student DNA experiments and is the world's largest provider of student lab instruction in molecular genetics.

The DNALC's *Biomedia* Group is one of the largest producers of Internet content on genetics and molecular biology. Its Internet portal, *Gene Almanac* (www.genealmanac.org), and family of content sites received 4.9 million visitors in 2003. These award-winning sites integrate design, usability, and function with authoritative science content.

Teaching and Learning Services to Secondary Schools and JC in Singapore

This DNA Science project presents an accurate analog of human genome research, which incorporates the highest aspirations of "hands-on" learning. Like genome scientists, students share common data and statistical analysis tools via Internet servers. The experience is entirely "open-ended" – each student's sequence result is unique, and each student uses database and statistical tools in novel ways to test his or her own hypotheses about the relatedness of human beings. In comparing their own DNA with that of other students and populations from around the world, students can learn about our shared ancestry – going all the way back to African ancestors. Entering their own data into the sequence database also encourages students to consider the uses and potential misuses of personal genetic data. We believe that this proposed DNA Science programme is the single best programme to help students understand genome technology – and to think about what it will be like to dwell in the gene age.

? ***Sequencing Service*** The *Sequencing Service* is the first venture to allow large number of students to see a bit of their own genomic DNA sequence. The *Sequencing Service* will be partially administered by JC and Secondary School teachers and students under the direction of a DNA Centre@NIE staff member, whose responsibilities include setting up sequencing reactions, precipitating dye-labeled DNA, loading sequencing gels, operating the DNA sequencer, and uploading the sequence files into the *Sequence Server* database.

? ***BioServers* Internet service** The *Genetic Origins* and *BioServers* Internet sites from our collaborator, CSHL, have all the information needed for students to perform the *Alu* and mt DNA experiments and analyze the results – including online protocols, reagents, animations and videos explaining key concepts, and database tools. CSHL is now developing new sites which will be used in this project:

Plant DNA science: *Greenomes* Internet site (www.dnalc.org/plants/)
Bioinformatics Calculator (www.dnai.org/genecalc/)
Gene Boy (www.dnai.org/geneboy)

We also plan to investigate genetic polymorphisms in animals, particularly pedigreed horses, dogs, and cats, by using standard STR markers. In addition, we will also train teachers and students how to identify GM foods by using DNA-based molecular methods.

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