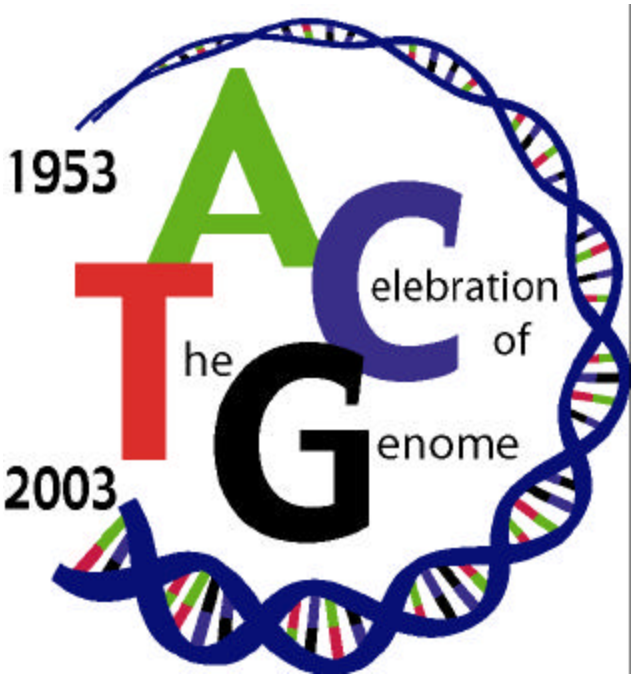

DNA Program Kit

Fifty Years of DNA
From Double Helix to Health

National Human Genome Research Institute *National Institutes of Health*
Department of Health and Human Services

United States Department of Energy

Science Museum of Minnesota *Association of Science and Technology Centers*

Acknowledgements

This project was the result of a relatively brief but rewarding collaboration between individuals at a number of public and private institutions. Its goal was to establish a link between science education centers and existing science research programs thus providing an avenue for sharing current research results with the public. The DNA Kits have received overwhelming support from museum participants, sponsors and donors. We hope that this project encourages other museum educators to seek out collaborations that foster current science initiatives.

Kris Wetterstrand and Jane Peterson at the National Human Genome Research Institute (NHGRI) in the National Institutes of Health (NIH) initially crafted the concept for this project when they began planning a series of events celebrating the essential completion of the Human Genome Project, the 50th -anniversary of Watson and Crick's description of the structure of DNA and the announcement of the new NHGRI plan for genomic research. They were interested in extending the celebration beyond the Washington, D.C. area and contacted Wendy Pollock at the Association of Science and Technology Centers (ASTC) hoping to illicit some help in attracting museums nationwide to participate in the event. Wendy contacted J. Newlin at the Science Museum of Minnesota because she knew they had been successful in developing hands-on biology exhibits and programs in a public institution.

A proposal for the development of DNA kits, which would contain a variety of activities and content information about DNA, sequencing and the Human Genome Project was drafted and funds allocated by NHGRI and various donors acknowledged here. The end result is this kit - full of information and fun ideas to use during the month of April, 2003 to help celebrate two simultaneous historical events, but can also be used throughout the year as an addition to public programming or education programs.

Our thanks to Kris, Jane and Wendy for initiating this exciting and worthwhile project. It has been a pleasure to work with each of you and we look forward to future collaborations and endeavors.

We are indebted to a number of individuals who helped to make the DNA Program Kits possible. At the Science Museum of Minnesota (SMM), the graphics team of Pam Ebersole, Brent Shipley, Craig Theisen, Jay Higashi and Lonnie Broden were responsible for creating and developing the graphic panels used in the kit. A huge thank you to all of the SMM volunteers who donated their time to assemble the kits, we would not have been able to complete this project on time without their help.

Bob Blakesley at the NIH Intramural Sequencing Center was gracious enough to provide expertise, photos and content information about DNA sequencing and the cystic fibrosis gene. Daryl Pritchard and Susan Vasquez of the NHGRI provided helpful comments regarding this manual. Bob Fulton at the Washington University Genome Sequencing Center and Lisa Marinelli at the Whitehead Institute/MIT Center for Genome Research

provided the capillary electrophoresis units that are part of the sequencing simulation activity.

Through the efforts of Guy Keyes, Cold Spring Harbor Laboratory Press has generously donated a number of books included with the kit.

We would like to thank the sponsors of this kit, Pfizer, IBM Life Sciences, Fisher Scientific International and Applied Biosystems, without whose support this undertaking would not have been possible. Additionally, the Foundation for NIH has worked closely with NHGRI to raise funds for all the activities of the "Celebration of the Genome".

A special thanks to Edvotek and specifically, Jeff Chirikjian who provided discounts on equipment and supplies, allowing us to provide you with state-of-the-art electrophoresis apparatus. Edvotek is a leader in providing quality educational materials and we are proud they have joined us in this initiative.

We hope you enjoy using the activities and equipment provided in these kits. It has been our pleasure to pull all of these resources together and hope the material allows you to gain some educational insight into genetics and the Human Genome Project. We apologize in advance for any errors or omissions and encourage you to contact us with any comments you may have.

Susan Fleming, PhD.
Project Leader, Human Biology
Science Museum of Minnesota
sfleming@smm.org

Ana Kaveh
Program Supervisor
Science Museum of Minnesota
akaveh@smm.org

J. Newlin
Director, Physical Sciences
Science Museum of Minnesota
newlin@smm.org

Table of Contents

Introduction

The original goal of the DNA Program Kit was to alert museum visitors to two historic events scheduled to occur in April, 2003 - the fiftieth anniversary of Watson and Crick's description of the structure of DNA and the essential completion of the Human Genome Project. That two such milestones in biological history should be linked in time is truly serendipitous and certainly worthy of a celebration.

The Science Museum of Minnesota has a long history of developing exhibits and programs that encourage independent exploration and discovery. With the opening of our new building in 1999, an entirely new gallery was formed called the Human Body Gallery. In this gallery, visitors use a variety of interactive tools to investigate the sometimes complex world of the human body. From the Bloodstream Superhighway, to the Body Hotel, the Sneezer exhibit and the Cell Lab, where visitors can put on a lab coat and carry out experiments just as a scientist would, visitors explore topics in human biology in a fun yet informative way.

For several years, we have worked on the development of biology programs and exhibits that engage visitors in topics long considered to be too difficult for hands-on exhibits, specifically cellular and molecular biology. Contrary to past expectations, visitors eagerly tackle complex subjects in biology and relish the opportunity to use authentic scientific equipment and supplies when given the proper tools. Museum visitors place value on doing "real science" in science centers. We have incorporated many of the techniques that we have found to be successful at the Science Museum of Minnesota into the DNA Program Kits. It is our desire that by introducing methods for engaging the public in activities about DNA, the Human Genome Project and its implications for the future, more museum educators and developers will try to incorporate current science into their museum programs. Collaboration with researchers and individuals at public and private research institutions provide tremendous resources and expertise.

It is not always easy to do real biology in a public museum. Many procedures in the biological sciences use toxic or hazardous chemicals, have results that are often difficult to see because they exist on a microscopic level, or happen too fast or too slow or use a vocabulary that overwhelms the average visitor. Developing successful programming in the biological sciences requires careful attention to detail, simplifying complex details, (but not "dumbing it down") and just allowing visitors to play around a little.

The DNA Program Kit provides you with a few tools to help ease your visitors into the world of genetics and genomics. Activities are geared for a variety of ages and abilities. You may find that you are comfortable with a certain set of activities and use those exclusively. Or, you may find that you like to mix it up a bit and use several of the activities during a single programming event. Most of the activities can be used together, in fact we encourage this, and it makes for a richer and more satisfying experience for the visitor.

We have tried to think of everything you will need for each of the activities, most everything is included in the kit. Those things that are needed and are not part of the kit are listed for each activity. You may need to purchase additional supplies to replenish some of the activities but most of these can be found in your neighborhood grocery store.

We highly recommend that you take advantage of the Educational Kit "The Human Genome Project-Exploring our Molecular Selves" during training sessions with staff and volunteers. The information is very good and the multimedia activities help to explain some of the more difficult concepts in genetics. The genetics timeline is a lot of fun to explore. If possible, make the contents of the kit available for visitors to use as well.

We are hoping that the kits will continue to evolve over the course of a year or so and there is even talk of creating a second generation of kit. Your input will be essential in the next generation of kits and crucial to the continued refinement and tweaking of the activities. Keep track of those things that you like, dislike or would like to change or modify.

We would like to have all those individuals who received the DNA Program kits attend a workshop in November, 2003 at the ASTC meeting in St. Paul, MN. Look for the announcement in your preliminary program and plan to attend if you will be at the meeting. If not, send us your comments and suggestions.

A. What is the Human Genome Project?

The Human Genome Project (HGP) is an international research effort to determine the DNA sequence of the entire human genome. Contributors to the HGP include the National Human Genome Research Institute (NHGRI) of the National Institutes of Health (NIH), which initiated its funding of the HGP in 1988; the U.S. Department of Energy (DOE), where discussions of the HGP began as early as 1984; numerous universities and laboratories throughout the United States; and international partners in the United Kingdom, France, Germany, Japan and China.

The essential completion of the HGP is an important milestone in the history of genetics research. In 1911, Alfred Sturtevant, then an undergraduate researcher in the laboratory of Thomas Hunt Morgan, realized that he could - and had to, in order to manage his data - map the locations of the fruit fly (*Drosophila melanogaster*) genes whose mutations the Morgan laboratory was tracking over generations. Sturtevant's very first gene map can be likened to the Wright brothers' first flight at Kitty Hawk. In turn, the Human Genome Project can be compared to the Apollo program bringing humanity to the moon.

HGP researchers deciphered the human genome using three tools: producing what are called linkage maps, complex versions of the type originated in early *Drosophila* research, through which inherited traits (such as those for genetic disease) can be tracked over generations; making maps that show the locations of genes for major sections of all

our chromosomes; and determining the order, or "sequence," of all the bases in our genome's DNA.

The HGP already has revealed that there are probably somewhere around 30,000 human genes. The existing and ultimate products of the HGP will give the world a resource of detailed information about the structure, organization and function of the complete set of human genes and other functional elements found in DNA. This information can be thought of as the basic set of inheritable "instructions" for the development and function of a human being.

The International Human Genome Sequencing Consortium published the first draft of the human genome in the journal *Nature* in February 2001 with the sequence of the entire genome's three billion base pairs some 90 percent covered at an accuracy of 99.9%. A startling finding of this first draft is that the number of human genes appears to be significantly fewer than previous estimates, which ranged from 50,000 genes to as many as 140,000. The essentially complete 'finished' version of the human genome is scheduled to be completed two years earlier than originally anticipated, in April, 2003. Sequence finishing requires much more time and resources than the draft sequence, because all possible efforts are made to close gaps and resolve difficult regions. This final version will be 99.99% accurate at the base pair level and 95% of the three billion base pairs will be represented.

Upon publication of the draft of the genome, in February, 2001, Francis Collins, the director of NHGRI, noted that the genome could be thought of in terms of a book with multiple uses: "It's a history book - a narrative of the journey of our species through time. It's a shop manual, with an incredibly detailed blueprint for building every human cell. And it's a transformative textbook of medicine, with insights that will give health care providers immense new powers to treat, prevent and cure disease."

The HGP also sponsors efforts to characterize the entire genomes of several other organisms used extensively in biological research, such as mice, rats, fruit flies and flatworms. These efforts support each other, because most organisms have many similar, or "homologous," genes with similar functions. Therefore, the identification of the sequence or function of a gene in a model organism, for example, the flatworm *C. elegans,* has the potential to help find and explain a homologous gene in human beings, or in one of the other model organisms. We can compare the landscape of the human genome with that of older species and identify evolutionarily conserved regions of DNA. This will allow us to identify sections of DNA that are functionally very important because they haven't changed over millions of years of evolution. The genes found in these regions of the genome may constitute some basic building blocks of life.

B. What are the future implications of the human genome project?

Ethical, Legal and Social Implications (ELSI)

The planners of the Human Genome Project (HGP) recognized that the information gained from mapping and sequencing the human genome would have profound implications for individuals, families and society. While this information would have the potential to dramatically improve human health, they also realized that it would raise a number of complex ethical, legal and social issues. How should this new genetic information be interpreted and used? Who should have access to it? How can people be protected from the harm that might result from its improper disclosure or use?

The Ethical, Legal and Social Implications (ELSI) Research Program was established to address these issues and has become an integral part of the HGP. ELSI - provides an approach to scientific research by identifying, analyzing and addressing the ethical, legal and social implications of human genetics research at the same time that the basic science is being studied. In this way, problem areas can be identified and solutions developed before scientific information is integrated into health care practice.

Individualized Medicine

In the last decade, a new research area called pharmacogenomics has arisen out of the information generated through the HGP. This area of study blends pharmacology with genomics, correlating information about individual responses to medical treatments with genetic data. Information gained from the HGP could potentially lead to development of drugs that are customized for a particular population.

Most drug reactions, and many of those that are fatal, are the result of adverse responses to the metabolism of a drug in the body. Enzymes in the liver (specifically P-450 type enzymes) are responsible for breaking down chemicals producing either potentially serious side effects or reducing the effectiveness of a drug's potential. Each individual may eventually be classified by genotype, allowing drugs to be tailor made according to the type of metabolic enzymes present. This type of customized drug delivery should reduce the number of doctor and hospital visits, resulting in a more efficient and cost effective medical system.

Genetic Tests

One of the most obvious applications of the information generated by the HGP is in predicting and diagnosing genetic diseases. Several hundred genetic tests are currently clinically available and their number is expected to increase dramatically in the next decade. Genetic tests that detect mutations in inherited genetic disorders, such as cystic fibrosis, Huntington's disease, Duchenne muscular dystrophies and sickle cell anemia are currently available. These genetic tests do not detect every mutation associated with a particular disease, but with the additional information provided by the HGP, the potential to detect even obscure mutations is possible.

Some genetic tests have recently become available allowing individuals to monitor and prevent certain types of cancer. Given a family history of breast cancer, colon cancer or

ovarian cancer, individuals can elect to be screened for the presence or absence of predictive mutations within their genotype. Information gained from these predictive tests can help physicians and patients manage and prevent the onset of disease.

C. Where are we going from here?

With the completion of the Human Genome Project, we are now entering a genomic era where we can expect to see great improvements in the treatment and prevention of disease. For example, disorders such as breast cancer and Parkinson's and Alzheimer's diseases result from a combination of environmental factors and multiple genes. The Human Genome Project will provide insight into what these genes are and how they interact with each other and with environmental factors. This will help lead to new ways to diagnose these diseases and devise strategies for prevention or treatment. By determining the genes involved in different diseases, we will develop a molecular approach for diagnosis and treatment of disease based on causes rather than symptoms. This approach will be more effective and precise than the current approach because doctors will be able to recognize conditions earlier and prescribe the necessary measures taken to avoid illness rather than waiting until you develop serious conditions and guessing how to fix the problem.

Other potential benefits of the HGP include advances in our knowledge of evolution and comparative genomics, migration of human populations through history, and improvements in DNA forensics, agriculture and bioprocessing. As we look to the future of health sciences we see a bright new horizon. For the first time in history, humankind can read its genome - its Book of Life. This book is unlike any other, for, in reading it, we will uncover an ever-expanding view of ourselves. - Francis Collins.

Proteomics

With the Human Genome Project complete, we will now be able to associate genes with the full set of proteins that make up and run the body. Many individual genes provide the instructions for generating multiple products. Our set of roughly 30,000 genes codes for even more proteins. Proteomics is the study of both the function and interactions of all human proteins. Our knowledge of proteomics will allow us to fully understand how gene products interact with one another to carry out and regulate all of the complex mechanisms of the body. The field of proteomics is advancing rapidly, and new technologies are being developed to rapidly study the processing and interactions of the complete set of proteins in cells.

What is Next for the HGP?

NHGRI is developing a new plan for the future of genomics and of the institute to be released in April, 2003. This plan focuses on three general themes: genomics and biology, genomics and health, and genomics and society.

D. What other events are planned for April, 2003?

- Proclamation of April 2003 as "**Genome Month**" and April 25th, the 50th anniversary of the publication that first described the structure of DNA, as "**DNA Day**."
- The opening in April 2003 of a preview of a **genomics exhibit,** "Genome: The Secret of How Life Works" at the Smithsonian Institution's Arts and Industries Building in Washington, D.C., produced by Clear Channel Communications with support from Pfizer, Inc. The full exhibit will open at the Smithsonian in June 2003 and then will tour nationally for five years.
- On April 14 and 15, a two-day **scientific symposium** at the NIH that will be satellite broad cast and web cast to institutions around the world. Participants, including James Watson, Francis Collins and members of the International Human Genome Sequencing Consortium, will reflect on the history of DNA since 1953 and describe the science of the Human Genome Project. Leading scientists whose work has been supported by a range of NIH Institutes and Centers will explore advances in biological research, medicine, health care, and disease research, that breakthroughs in genomic science make possible, and will include the unveiling of the NHGRI's new scientific plan.
- In the morning of the 15th, a half-day **public symposium** will be held at the Smithsonian's National Museum of Natural History. The talks will be designed to convey how genomics influences health and society and will be satellite broad cast and web cast to institutions around the world. Speakers will address issues such as the effect of the Human Genome Project on medicine and the media's view of the genome.
- If you would like to access a satellite broadcast of either of these symposia please send a message to apr2003@mail.nih.gov. To view the web casts 'live' or after the symposia take place, please visit the NIH Videocast web site at http://videocast.nih.gov/.
- Schools and museums throughout the country can utilize such tools as an **educational event** including James Watson, Francis Collins and high school biology students taped on April 15th, and made available via the NHGRI web site (http://www.genome.gov/education): on "DNA Day", April 25.
- Resources currently available on the NHGRI web site (http://www.genome.gov/education): Information regarding the American Society of Human Genetics Mentorship Program, a network of genetics/genomics professionals available for outreach activities in your area and lesson plans designed to accompany the NHGRI multimedia education kit.

Kit Contents

	Description		Vendor	Catalog #	Vendor Info
DNA Model Building	K'Nex		K'Nex		K"Nex Industries Kathryn Needhan
		Instruction Guide	Science Museum of Minnesota (SMM)		
	Pop Beads	White 5 way(280)	Carolina	RG 17 1041	Carolina Biological 800-334-5551
		Red (280)	Carolina	RG 17 1043	"
		Green (280)	Carolina	RG 17 1044	"
		Yellow (280)	Carolina	RG 17 1045	"
		Blue (280)	Carolina	RG 17 1046	"
		Orange (280)	Carolina	RG 17 1047	"
		Purple (280)	Carolina	RG 17 1048	"
		Clear connectors (300)	Carolina	RG 17 1049	"
		Wavy (280)	Carolina		"
	Containers for beads		Fisher	02544213A (case of 100)	Fisher Scientific 800-766-7000
	Container for K'Nex		Fisher	02544214A (case of 100)	"
	Molecular model		Ward's	81 D7005	Wards
DNA Extraction	Wheat germ		Cub Foods	Must be raw, not toasted	
	100 ml graduated cylinders (2)		Daigger	MX6856D	Daigger 800-621-7193
	Solo plastic cups (12)		Target		
	Measuring spoons		Target	½ Tbls, ¼ teas.	
	Baking soda		Cub Foods		
	Meat Tenderizer		Cub Foods	Adolph's unseasoned	
	Liquid detergent		Cub Foods		
	Plastic spoons (12)		Cub Foods		
	5x150 mm stirring rods (12)		Carolina Biological	RG 71 1305	Carolina Biological 800-334-5551
	17X100 tubes (60)		Fisher	14-956-8J	Fisher Scientific 800-766-7000
	Squeeze bottles (2)		Daigger	MX2757D	"
	15 cm plastic droppers (60)		Daigger	MX20410A	"
	Test tube rack		Daigger	MX2510	"
	50 ml centrifuge tubes		Daigger	MX4880HX	"
	Visitor guide		SMM		
Electrophoresis	Electrophoresis apparatus		Edvotek	515, Model M36	Edvotek 800-338-6835
	Power supply		Evotek	503	"
	Micropipet		Gibbco Scientific	Labpipette, 25 µl fixed volume or 10-100 µl adjustable volume	Gibbco Scientific Mark Gabel 612-786-0880
	Micropipet tips (box of 96)		Daigger	MX2051B	Daigger 800-621-7193
	50X TAE (50 ml)		Edvotek	607	Edvotek 800-338-6835

	Microfuge tubes (30)		Daigger	MX4264RA	Daigger 800-621-7193
	Electrophoresis samples		SMM		Food coloring, glycerin and water
	Agarose (10 gm)		Edvotek	605B	Edvotek 800-338-6835
	Foam tube holder		Daigger	MX21645A	Daigger 800-621-7193
	Plastic bottle	Wide mouth	Daigger	MX24250A	"
		Narrow mouth	Daigger	MX24251A	"
Sequencing Simulation	Capillary electrophoresis unit		NIH Sequencing Centers		
	Graphics	Sequencing procedure	SMM		
		DNA sequencer traces	SMM		
	Washable markers		Target		
Sequencing Analysis	Cystic fibrosis sequence	Genomic sequence	SMM		
		Protein sequence	SMM		
Genetic Traits	PTC tasting paper		Carolina	RG174010	Carolina Biological 800-334-5551
	Poker chips		Target		
	DNA sample	Herring sperm DNA	Sigma	D1501-1g	Sigma Chemical 800-325-3010
		Plastic vials	Daigger	MX28359B	Daigger 800-621-7193
Books	*Baaa* by Cynthia Pratt Nicolson		Kids Can Press		
	Cell Division and Genetics by Robert Snedden		Heinemann Library		
	Microbes, Bugs and Wonder Drugs by Fran Balkwill, Mic Rolph, Victor Darley Usmar *Enjoy Your Cells, Germ Zappers, Have a Nice DNA and Gene Machines* by Fran Balkwill, Mic Rolph		Cold Spring Harbor Laboratory Press		www.cshlpress.com
Chromosome map	Landmarks of the Human Genome		US Department of Energy, Office of Science		www.ornl.gov/hgmis
NHGRI Educational Kit	The Human Genome Project: Exploring our Molecular Selves		National Human Genome Research Institute,(NHGRI),National Institutes of Health (NIH)		
Packing Trunk			Pelican	1650F	CDP Industries 800-882-4730

DNA Model Building

DNA is an incredibly complex yet simple molecule. Its structure is like a twisted ladder with rungs made of nucleotides and a backbone of phosphates. Using three different types of models, visitors have the opportunity to build their own DNA molecules and visualize the three dimensional structure of DNA.

DNA Model Building At a Glance

Goal	Allow visitors to construct their own DNA models
Level	Introductory to advanced
Age	8 and above (younger with adult assistance)

When James Watson and Francis Crick published their seminal paper (see appendix I for full text) describing the structure of DNA fifty years ago, no one could have anticipated the enormous impact it would have. Scientists had been struggling for years to put all of the pieces together and solve the DNA puzzle. Many others were close; Linus Pauling and Robert Corey were convinced that DNA was a helical structure, but had proposed a helix with three intertwined strands[1]. Sven Furberg had the correct orientation with the phosphates on the outside and the bases inside parallel to each other, but proposed a single stranded helical structure[2]. Watson and Crick were clever enough to assimilate all of the experimental data and correctly propose the double stranded alpha-helix structure. This eventually resulted in the awarding of a Nobel Prize in Medicine and Physiology to Watson, Crick and fellow collaborator Maurice Wilkins in 1962. Rosalind Franklin was another important contributor toward the elucidation of the structure. She produced the x-ray data that Watson and Crick used during their final analysis. Unfortunately, she died before the Nobel Prize was awarded and has only recently been acknowledged for her contributions.

Elucidating the structure of DNA proved to be an elusive endeavor for many scientists. It seems so logical now and one can only be amazed by its basic though elegant physical characteristics. It is composed entirely of only four different molecules, arranged within a helical structure that allows for replication, transcription and translation. The precise arrangement of these four molecules contains all of the information necessary to control development of different cell types and differentiation between species. We already have the sequence of numerous model organism genomes and by April, 2003 we will know the sequence of DNA in the human genome. We have certainly come a long way in just fifty years.

[1] Pauling, L., and Corey, R. B., Nature, 171, 346 (1953); Proc. U.S. Nat. Acad. Sci., 39, 84 (1953).
[2] Furberg, S., Acta Chem. Scand., 6, 634 (1952).

Let's review with a little background information:

Cells are the basic building blocks of every living system and can be compared to a small city. Each cell is able to produce energy, metabolize and process food, dispose of wastes, carry out cellular functions and interact with the surrounding community.

Like a library within a city, each cell has a nucleus, containing all of the instructions for directing the activity of the cell. A library contains reference books, while a nucleus has **DNA** or deoxyribonucleic acid. Unlike DNA, reference books are written with 26 different characters. DNA only uses four characters or nucleotide bases; adenine (**A**), guanine (**G**), cytosine (**C**) and thymine (**T**). If you opened up a DNA reference book, it would read like this: AGTTCTGCATCCTGGATCCA….. hard to read unless you are good at cracking secret codes.

In a reference book, each chapter describes a particular topic. **Genes** are like chapters within DNA. They are units of DNA that contain specific sequences coding for specialized proteins or functions. Genes and other elements that control gene function are spread out over the entire length of DNA in a cell. It is estimated that the human genome contains approximately 30,000 genes.

A library contains several different types of reference books, The Encyclopedia Britannica, A Readers Guide to Literature, Who's Who in Science, Dictionaries, etc. Each human cell (except red blood cells, which lack a nucleus) has 23 pairs of reference books or **chromosomes**. Most organisms have duplicate copies of chromosomes in case one set gets damaged or lost. Human chromosomes are typically labeled according to size, with one being the largest and chromosome 22, being the smallest. Chromosomes range in size from 50 million to 263 million bases.

The entire collection of reference books found within a library is like a **genome**. Each cell in the human body contains a complete set of instructions in the nucleus called the human genome. Genomes vary widely in size depending upon the organism. Bacterial genomes contain about 600,000 bases while human and mouse genomes are made up of over 3 billion base pairs. **The Human Genome Project** is an attempt to decipher the sequence of bases in human DNA as well as that of other model organisms. It is like reading all of the reference books in the library cover to cover.

Whether we like it or not, reference books are never allowed to leave the library, and the same is true of DNA. It never leaves the nucleus. In order for information to be transmitted to other parts of the cell, the information must be copied. The nucleus does not contain a copier machine but uses a process know as **transcription**. DNA is transcribed into **RNA**, or ribonucleic acid. RNA is exactly like DNA with a few minor exceptions: RNA is single stranded and instead of the nucleotide base thymine (T), uracil (**U**) is used. RNA can travel out of the nucleus to other parts of the cell, and the information is used to assemble **proteins**. Proteins are like the building blocks of the city, they form structures inside and outside of the cell and carry out many of the cellular functions.

DNA is found in every living organism. It's in the food we eat, the trees that shade us from the sun and that we use to build our houses, the bacteria that can make us sick or help us digest our food and the organisms that help make bread rise. The arrangement of nucleotides within DNA has been changing in all species leading to evolution between species as well as **genetic variation** between organisms within a species.

The structure of DNA is often compared to a ladder. The outside of the ladder is made up of sugars and phosphates linked together to form a backbone. Hanging off of the backbone like beads on a string are the bases (A,T,G and C). These bases form very strong attachments with the bases on the opposite strand of DNA by hydrogen bonding. These attachments form the rungs of the ladder and occur in a very specific manner. Adenine (A) will only bond with thymine (T) on the opposite strand, and guanine (G) will only form a hydrogen bond with cytosine (C). This specific bonding is commonly referred to a "base pairing". Whenever you find an A on one strand, a T should be across from it. Whenever there is a G on one strand, a C should be across from it.

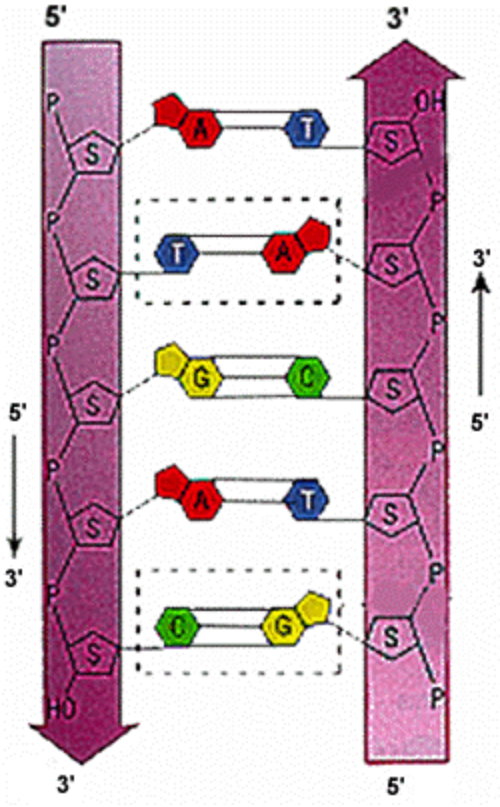

Figure 1. Structure of the DNA helix. Reprinted from Biochemical, Biological and Genetic Connections, Student Interactive Tutorials, http://www.cs.stedwards.edu/~kswank/Dnabasepairs.html

The DNA ladder is twisted one and a half times to form a helix. This allows the molecule to coil into a very compact shape. During replication and transcription, the DNA unwinds so that the strand(s) can be copied. If you are interested in more information on DNA please refer to either the books included in this kit, the educational CD Rom prepared by NIH or the informational sheet distributed by the Department of Energy (DOE).

This DNA Program Kit contains three different DNA models, each of which illustrates an important characteristic of DNA.

1. K'Nex model – a very easy model to use and provides a good opportunity to demonstrate the helical structure of DNA. The K'Nex model has four different colors of connectors that can be used to represent the four nucleotide bases, but it is difficult to see the base pairing. This model can be used with children as young as five to seven years old.
2. Pop Bead model – this is an excellent example of the base pairing and ladder structure of DNA. You will need very strong fingers the first few times it is used. Until the beads are "broken in" we do not recommend using this model with children under ten years old. Once the beads are easy to snap together it can be used with younger children.
3. Molecular model – this illustrates the fine chemical structure of DNA and the molecular interactions involved in forming the alpha helix. This model can be assembled and painted by groups of students, volunteers or staff personnel. Once it is assembled it can be used as a permanent display.

You can choose to use all or just one of the models with your visitors. We highly recommend that you practice using both the K'Nex and Pop Bead models **before** using them in public demonstrations.

A. K'Nex Model

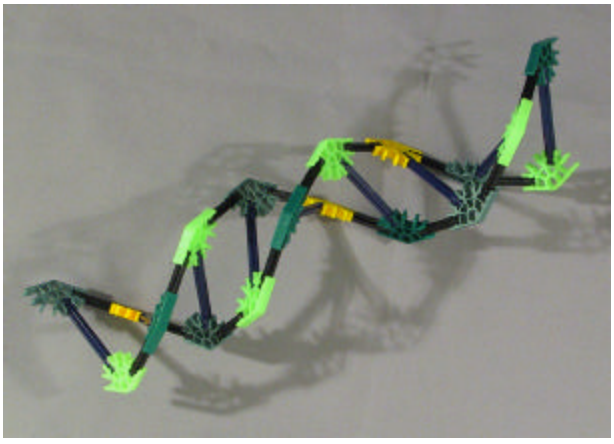

Figure 2. K'Nex DNA model

Equipment and Supplies

Short black pieces
Long blue pieces
Yellow, green, gray and fluorescent green connectors
Instruction guide for making a DNA helix using K'Nex pieces

Activity set-up

1. Remove the model pieces from the trunk.
2. Assemble a small DNA model using the instruction guide.
3. Place the instruction guide out with the model pieces

Things to think about when using this activity

- In our experience, boys are much more comfortable working with the K'Nex model than girls tend to be. Once girls get started however, they are enthusiastic about the K'Nex model, but may need some encouragement to put the first few pieces together.
- Don't worry about making visitors assemble their DNA models exactly as they should be. Allow some artistic interpretation, they will learn something about the structure of DNA no matter what they do.
- Even though the K'Nex model comes with four different colors for the connectors, the colors are so similar it is difficult to see the specific combinations. Don't worry if visitors randomly incorporate the colored connectors. You can explain the specific base pairing, and your model should reflect the base pairing, but visitors may or may not construct their models with the correct base pairing. You can reinforce the concept of base pairing using one of the other models or the sequencing activities.
- Encourage visitors to help in putting the models away. This is a fun activity for visitors of all ages and makes the job go much quicker.

Activity clean up

1. Take all pieces apart and place into their individual containers, except those models you would like to keep as examples.
2. Replace the lids to the containers, making sure that they are on tight.
3. Store model pieces in the program trunk with the instruction guide.

B. Pop bead Model

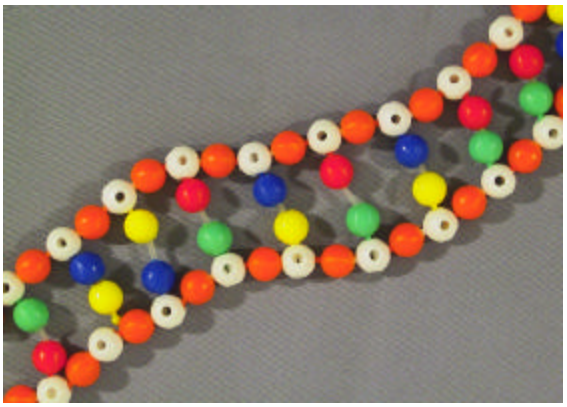

Figure 3. Pop bead DNA model

Equipment and Supplies

White 5 way beads (represent the phosphates)
Orange beads (represent the sugars)
Red beads (represents thymine)
Green beads (represent adenine)
Yellow beads (represent guanine)
Blue beads (represent cytosine)

Things you will need that are not supplied with this kit

Very strong fingers to put the pop bead model together

Activity set-up

1. Remove the beads from the trunk
2. Place plastic connectors in the red, green, blue and yellow pop beads. This is the most difficult part of putting this model together. It is a good idea to have this done before you use the beads with visitors.
3. Assemble a small piece of the DNA model as demonstrated in the figure below.

Things to think about when using this activity

- The pop beads are very difficult to put together the first time you use them. You will have sore fingers from trying to snap them together. Some of the beads are easier than others. If you find a stubborn bead that refuses to snap into place, try expanding the opening by inserting a ball point pen or the tip of a scissors into the hole. Press down, enlarging the opening and making it easier to insert the next bead.
- We highly recommend using your pop bead set with staff, volunteers or students **before** you use them for visitor demonstrations. **The more you use the pop beads, the easier they become to snap together.**

- Because of their size, the Pop beads could pose a choking hazard for young children. For this reason, we do not recommend allowing visitors to use the beads as an unsupervised activity.
- Place the plastic connectors in the beads that will be used as nucleotides (red, green, yellow and blue). Visitors can use these joined pieces when constructing their models. Leave the connectors in the beads when disassembling the models so they are ready for the next use.
- Encourage visitors to help in putting the models away. This is a fun activity for visitors of all ages and makes the job go much quicker.
- The base pairing in the helix can be color coded to correspond with the results the other activities in the kit:

Red beads (T) : Green beads (A)
Blue beads (C) : Yellow beads (G)

Activity clean-up

1. Take all pieces apart and place into their individual containers, except those models you would like to keep as examples. Leave the plastic connectors in the nucleotide beads to be used again.
2. Replace the lids to the containers, making sure that they are on tight.
3. Store model pieces in the program trunk.

C. Molecular Model

Equipment and Supplies

Model pieces Instruction guide
Paint

Activity set-up

1. Follow the instructions for construction of the model
2. Paint the model before using it in public demonstrations

Things to think about when using this activity

- This model should be assembled by staff , volunteers or better yet through a classroom activity. It is an elegant, yet relatively sturdy model that demonstrates the fine chemical structure of DNA. Volunteers and staff can use this model to initiate thought provoking instructions about DNA structure, function and analysis.
- Once the model is assembled, it cannot be stored in the program trunk, Before assembling the model, think about where it will be stored before it is completed. Because of its more delicate nature, we do not recommend placing it in an unsupervised area, otherwise you will be spending a great deal of time repairing your DNA structure.

Questions that may come up about these activities

How long is DNA?
If the DNA inside one cell was stretched out end to end, it would be approximately six feet long. It is estimated that an adult human has 60 trillion cells. That is a lot of DNA!

It is estimated that the human genome (that's all of the DNA in one cell) contains roughly 3 billion base pairs. If you construct a DNA model out of pop beads containing 12 bases pairs (or rungs of the ladder) it is approximately one foot long. If you constructed a model representing the entire human genome using pop beads, it would have to be 250 million feet long, or 47, 348 miles long. One fun activity to add to the DNA modeling station would be to represent this length using string and a map. Chromosome 1 is thought to contain 263 million bases pairs – 263 million base pairs/12 base pairs per foot = 22 million feet or 4150 miles. This is approximately the distance across the entire United States.

Where can I buy Pop beads for my classroom or home?
The beads in this kit were purchased from Carolina Biological. We have found other beads at Wal-Mart and some specialty toy stores.

D. Make and take ideas

This is a protocol from a 7th grade science teacher – (Kris Swartchick, Wayzata Central Middle School, Wayzata, MN). We have included if you want to have an activity allowing visitors to take something home. It is relatively inexpensive and easy to do.

Supplies needed:

Two 24-inch pieces of fishing line or string
Eight 2-inch pieces of blue chenille stems (pipe cleaners)
Eight 2-inch pieces of yellow chenille stems
Eight 2-inch pieces of red chenille stems
Eight 2-inch pieces of green chenille stems
Eighteen pieces of dried pinwheel pasta
Sixteen pieces of dried ziti cut pasta

1. Tie the end of a string onto a piece of pinwheel pasta.
2. Thread the string through a piece of ziti pasta followed by another piece of pinwheel pasta.
3. Continue alternating ziti pasta with pinwheel pasta until you have nine pieces of pinwheel pasta and eight pieces of ziti pasta.
4. Tie the string onto the last piece of pinwheel pasta so the pieces do not come off.
5. Repeat the procedure with the other piece of string.
   The pasta strung onto the two lines represent the sugar-phosphate backbone of DNA.

6. Lay the two pasta strings side by side.
7. Using **one** of the pasta strings, twist the ends of the chenille stems onto the pinwheel pasta.
   You can add the chenille stems in any combination you want, creating your own genetic code.

8. Repeat with the other strand, adding the colors on the opposite strand using the following base pairing:
   Adenine (red) always pairs with thymine (green)
   Guanine (yellow) always pairs with cytosine (blue)

9. Twist the ends of the chenille stems together creating the rungs of the DNA ladder.
   The rungs of the ladder represent the hydrogen bonds that hold the DNA strands together.

10. Holding your model up, give it a twist to demonstrate the helical structure.

DNA Extraction

A. DNA Extraction from Wheat Germ

DNA is the thread of life. Encoded in its genetic sequence is the information that makes each of us unique. In this DNA extraction activity, visitors are able to see long, stringy strands of DNA from raw wheat germ wrapped around a glass stirring rod.

DNA Extraction At a Glance

Goal	Isolate DNA from raw wheat germ or cheek cells
Level	Introductory
Age	8 and above (younger with adult assistance)

In the past several years, the DNA sequence of yeast, fruit fly, bacteria, humans and other species have been determined. Analysis of these sequences indicates that there is significant sequence similarity between different species. We as humans are not as genetically distinct from our most primitive ancestors as once assumed. Although we may not resemble a grain of wheat, the DNA from raw wheat germ is identical in physical and chemical composition to the DNA found in the cells of the human body. Visitors who isolate DNA from raw wheat germ can actually see what extracted DNA looks like.

This procedure is a simplification of a technique used routinely by research scientists to isolate DNA from plants, animals, yeast and other sources. The wheat germ cells and the nuclear membrane are broken apart by soap, releasing nucleic acids, proteins and other components into a solution. The mixture is stabilized by adding a bicarbonate buffer and. digestive enzymes are added to chew up the proteins that hold the DNA tightly coiled into chromosomes and to destroy those enzymes which break DNA into small pieces. Alcohol is carefully layered on top of the wheat germ mixture in order to precipitate the DNA. A glass rod swirled at the alcohol/water interface will pick up a thick, gooey blob of DNA. The thick blob consists of thousands of DNA strands wrapped around each other. Ordinarily, DNA is colorless, but when you wrap many DNA strands together it appears white.

The DNA Extraction from wheat germ activity is a very easy experiment for visitors of all ages. It does not require the use of sophisticated equipment and all of the reagents and supplies are available in grocery or hardware stores. One word of caution however: **raw wheat germ must be used for this protocol. Toasted wheat germ will not work.** Raw wheat germ can be found in the bulk food section of supermarkets or at a neighborhood Co-op. The experiment typically takes approximately 10-15 minutes to complete and is a good activity for large school groups.

Equipment and Supplies

Raw wheat germ
Distilled water
Liquid Hand Soap in a pump dispenser
Meat tenderizer
Baking soda
Plastic cup
Squeeze bottle for alcohol
Test tube rack
Measuring spoons (1 large spoon-1/2 Tablespoon, small spoon- ¼ teaspoon)
Mixing spoon
Glass stirring rod
Plastic dropper
100 ml graduated cylinder
15 ml plastic tube
Visitor guide

Things you will need that are not supplied as part of this kit

Bottles or small jugs containing distilled water
Waste bucket for wheat germ solids and alcohol mixture
Alcohol (either 70% isopropanol or 95 % denatured alcohol)
Tub or container to hold dirty cups, tubes, spoons and stirring rods

Activity set-up

1. Remove the supplies from the box, place on the table.
2. Fill squeeze bottle labeled "Alcohol" with denatured alcohol or 70% isopropanol.
3. Place plastic tubes in the rack.
4. Place one plastic dropper in each test tube.
5. Fill small water bottles with distilled water.

Things to think about when using this activity

- Each cup of wheat germ solid mixture produces enough solution to allow three or four visitors to extract DNA on their own. Encourage groups of participants to work together when preparing the solution, but allow them to each spool their own sample of wheat germ DNA.
- This kit contains enough supplies for approximately 60 visitors to spool DNA during one demonstration. You may need to clean and restock supplies if you plan to have more than 60 visitors.
- This activity has one section where visitors are waiting for the wheat germ solids to settle before spooling their DNA. This takes about 5 minutes and is a good opportunity to engage visitors in a discussion of DNA, the Human Genome Project or other topics. It is suggested that you have some other props available to use during this wait period, such as the DNA model
- We have found that wooden coffee stirrer sticks instead of glass stirring rods work just as well to spool the DNA. They can not be reused but are a good option when avoiding the use of glass

Procedure

1. Using the graduated cylinder, measure out 100 mls of water and pour it into the plastic cup.
2. Add 1 large spoonful of wheat germ to the water and mix using a plastic spoon.
3. Add one pump of liquid soap, stir for 1 minute.

   You will notice that the mixture will get very thick. This is because the cell membranes are broken apart by the soap, releasing nucleic acids, proteins and other components. The DNA makes the solution very viscous.

4. Add 1 small spoonful of meat tenderizer and 2 small spoonfuls of baking soda. Stir to mix.

   The meat tenderizer chews up the proteins which hold the DNA tightly coiled inside the cell and destroys the enzymes which break DNA into small pieces. We want the DNA to be long stringy strands, so it will wrap around the glass rod. The baking soda acts as a buffer system and brings the pH up to 8.0. This helps proteins separate from DNA.

5. Stir for 1 minute. Allow the wheat germ to settle to the bottom until almost all the wheat germ solids are on the bottom of the cup. This should take about 2-3 minutes.
6. Once the wheat germ has settled, use the plastic dropper bulb and transfer two or three **droppers full** of the wheat germ liquid at the top of the cup to a tube. Some of the wheat germ solid may transfer over to the tube; that is okay, but you don't want all the wheat germ to contaminate your solution.
7. Using the squeeze bottle containing alcohol, dribble alcohol down the side of the tube. Add an amount of alcohol equal to the wheat germ liquid.

   Try not to mix the two layers. Let the tube sit for a minute or two. You will see large and small bubbles appear at the interface between the two layers. This is the DNA, it does not dissolve in alcohol.

8. Carefully swirl a glass stirring rod at the interface of the two layers using small circles to spool or wrap the DNA around the rod. If you keep swirling and are careful not to mix the two layers, you will be able to pull out a big wad of DNA.

   The wad of DNA that you have collected on the rod is composed of millions of DNA strands. When many of these strands are swirled together they make a large sticky, slimy glob. Because this DNA sample has been purified very quickly, there are proteins and long chain sugars mixed in with the DNA Scientists use a similar procedure to produce highly purified DNA. You can touch your DNA sample to see how it feels.

9. When you are finished, scrape your DNA clump off the stirring rod into the cup and place all the liquids in the disposal bucket.
10. The plastic cup, tube and stirring rod can be placed in the plastic tub.

Activity clean up

1. Replace covers on all reagents and return to storage box.
2. Remove alcohol from squeeze bottle, store in non-flammable container.
3. Clean all cups, tubes, plastic pipets and stirring rods.
4. Dispose of liquid wheat germ waste using a janitorial drain. Sink drains with a trap may become clogged with wheat germ solids over time.

Questions that may come up about this activity

Can I do this at home?
All of the reagents used in this extraction are found either in a grocery store or local hardware store. The alcohol used is toxic if ingested, so caution should be used around young children or very immature teens. This experiment bench is a wonderful way to introduce visitors to complex topics such as DNA using familiar products and supplies. One of the wonderful applications of this activity is that participants use everyday items in unfamiliar ways. Who would think that liquid hand soap dissolves cell membranes or that meat tenderizer contains an enzyme called papain that can chew up other enzymes? It is a thrill for many visitors to actually see strands of DNA. Most individuals are unaware that the foods they eat contain DNA and that DNA is so important for life.

What can go wrong?
One of the most satisfying aspects of this protocol that it is essentially fool proof and almost every single participant ends up with a large snotty wad of DNA. Visitors want very much to succeed in their investigations and assume they did something wrong when things don't turn out. The DNA extraction from wheat germ very rarely fails **except when raw wheat germ is not used.** By far the single most common reason for failure is not using raw wheat germ but rather prepared toasted wheat germ (such as Kretchmers). Raw wheat germ can found in the bulk food section of most grocery stores and cooperatives.

How do I know this is DNA on the end of the stirring rod?
One of the drawbacks of this procedure it that many visitors want to know more. Frequently a visitor will ask "How do I know this is DNA?" or "If I look at this under a microscope will I see the double helix?". They would like some proof that this is indeed DNA on the end of the glass rod. We have investigated a number of options which would allow visitors to actually see that they have indeed isolated DNA, but none of these were successful. The protocol used in this activity is a very quick nucleic acid isolation and plant material traditionally has a very high proportion of polysaccharides

and other contaminants. We think that these contaminants prevent analysis of the product by conventional methods.

As a result, staff and volunteers can explain to visitors that under more careful conditions which limit contamination by other cellular macromolecules, the DNA sample could be analyzed and confirmed to be DNA. Although it would be wonderful to see, the DNA double helix cannot be observed under even the most powerful electron microscope. Seeing the structure of DNA requires the use of x-ray diffraction to see the double helix pattern.

How can I make this experience more valuable to the visitor?
Throughout this activity, volunteers should try to explain the process at each step, so visitors have a better understanding of what they are doing and why. Through training, staff and volunteers should be able to answer any questions and encourage insightful discussions about DNA, genetics, the Human Genome Project and genetic testing. This activity is a perfect opportunity to open up lines of communication with visitors about topics such as genetic diseases and genetically modified foods.

B. DNA Extraction from Cheek Cells

Developed by The New York Hall of Science, modified by Science Museum of Minnesota
(Adapted from a protocol by Judy Schleppler, SUNY Stonybrook)

This is an activity that we have used with visitors for many years. It is a very easy procedure but one in which not all visitors will successfully isolate their own DNA. Those visitors who vigorously swish and remove lots of check cells will see some DNA, while others just don't collect as many cells and will not be able to see the DNA strands. We have noticed those individuals who are chewing gum, or have just eaten will not be as successful in extracting DNA. We think they have swallowed all of their available cheek cells and so not do not have as many available for extraction.

Regardless of the result, many visitors enjoy attempting to extract their own DNA sample. We often let them take home their sample in a small microfuge tube, especially when this activity is carried out as part of a classroom activity. One word of caution, however, saliva is used in this activity, so all tubes, tips and cups should be disinfected either by autoclaving or rinsing in a 10% bleach solution.

Equipment and Supplies

50 ml tube with cap
Liquid hand soap in a pump dispenser
Plastic disposable pipet
Squeeze bottle
Glass stirring rod
1.5 ml microfuge tube

Things you will need that are not supplied as part of this kit

Dixie cups
Tap water
Containers for disposing of cups, tubes, plastic pipets and stirring rods
Alcohol for precipitating DNA
Extra microfuge tubes (if samples are to be taken home)

Procedure

Collect cheek cells

1. Swill 5 ml of water in your mouth for 30 seconds. It is better if you vigorously swish the water back and forth across your mouth.
2. Spit the water and cheek cell mixture back into the Dixie cup, and then pour the mouthwash into a 50 ml tube.

The swishing washes cells from the inside of your cheeks into the water. More vigorous swishing washes more cells off and yields more DNA. We *are also washing bacterial cells from the inside of the mouth, so will isolate their DNA as well.*

Release DNA from inside the cheek cells

3. Add one pump of liquid detergent.
4. Cap the tube and mix the contents by gently inverting several times. Do not shake the tube or you will get lots of foam.

The detergent removes the greasy cell membranes from around the cheek cells (and bacterial cells). The cheek cells also have a membrane around the nucleus, which is also removed. The cell contents (including the DNA) are released into the detergent solution.

Precipitate the DNA

5. Remove the cap and carefully add 4 ml of 95% alcohol using the squeeze bottle. Place the tip of the alcohol bottle against the side of the 50 ml tube and dribble the alcohol into the tube. The alcohol should layer on top of the mouthwash solution.
6. Hold the tube still for 1 minute. Look for the clouds of white, cottony strands of DNA forming between the alcohol and salt water layers.

DNA is not soluble in ethanol, so it precipitates where the ethanol and salt water layers meet. Most other cell materials remain in the salt water layer. Bubbles displaced from the salt water get trapped in the DNA strands and make them easier to locate. Single DNA molecules are way too small to see-a strand seen here is probably a clump of thousands of DNA molecules.

Collect the DNA

7. Drop a glass rod into the tube and let it rest on the bottom.
8. Wind the DNA onto the rod by turning it in one direction. If you stir the rod in the tube, the DNA may stick to the rod. If the DNA does not stick to the glass rod, use a plastic pipet to carefully remove your DNA.
9. Scrape the DNA into a small microfuge tube and add alcohol. You can take this home if you like. For long-term storage of your DNA, store your tube in a freezer.

Measurements and Micropipetting

Many scientific procedures require the measurement of very small liquid volume amounts. Measurement of these small liquid volumes involves the use of a digital micropipet capable of measuring as little as one microliter (ml), or one millionth of a liter. This activity describes the proper use of a micropipet.

Micropipetting At a Glance

Goal	Learn how to use a micropipet
Level	Introductory
Age	5 and above (younger with adult assistance)

Adjustable volume digital micropipettors typically come in three different sizes: small-volume pipettors (0.5-10 µl), mid-range micropipettors (10-100 µl) and large-volume pipettors (100-1000 µl). Either a fixed volume (25 µl) micropipet or a midrange (10-100µl) adjustable volume micropipet is supplied with this kit. Both types of micropipets use the mid-range disposable plastic tips (10-100 µl) which are usually yellow in color. Micropipets are very easy to use but a few precautions should be observed when using this instrument:

- Never use the micropipettor to withdraw liquids from a sample without a tip in place. This could ruin the piston and the sealing gasket.
- Never invert or lay the micropipettor down with a filled tip; fluid could leak into the barrel of the pipettor and destroy the piston and seal.
- Never let the plunger snap back after withdrawing or expelling liquids. This could damage the piston.
- Never immerse the end of the micropipettor in fluid.
- Never flame the tip of the micropipettor.
- In a laboratory setting, never reuse a tip that has been used in a different reagent.

You will notice that the micropipet supplied with this kit is a laboratory grade instrument. Visitors appreciate using real scientific equipment rather than cheaper imitations. This is a very expensive part of the kit and should be handled with the utmost care. If at any time the micropipettor fails to withdraw or hold solutions in the tip, the gasket and seal may need to be cleaned. We recommend contacting a local laboratory supplier to arrange for maintenance. Do not take the micropipet apart yourself, unless you have experience cleaning and repairing these instruments. Many universities and research institutions have maintenance contracts for micropipets. They may be able to help you in servicing your micropipet.

This activity is an easy exercise for all ages and takes about 5-10 minutes to complete. If you are interested in adapting this activity for large groups we recommend either locating

more micropipets (through discards from your local university), purchasing disposable glass capillary pipets and plungers through Carolina (Wiretrol micropipets, RG21-1156) or using the disposable plastic pipets. You can measure small volumes with the disposable plastic pipets by squeezing the barrel of the pipet rather than the bulb.

Equipment and Supplies

25 µl fixed volume micropipettor
Box of plastic yellow pipet tips
Tubes of colored water
Tip disposal cup or a cup of water for rinsing used tips
Small microfuge tubes for expelled volumes

Things you will need that are not supplied as part of this kit

A cup for disposal of used tips **or** a cup of water to rinse used tips if you plan to use them again.
Tubes or small samples of colored water.

Activity Set-up

1. Remove the micropipettor, tips and small microfuge tubes from the kit.
2. Prepare a few samples of colored water in small tubes or vials.
3. Have a cup available for disposal of tips or filled with water for rinsing used tips.

Things to think about when using this activity

- The micropipet supplied in this kit is very expensive. Please do not let visitors mistreat the pipet in any way. We have found that visitors are very respectful of equipment that looks scientific. They tend to be more serious when using the real thing.
- This activity can be used during training to help familiarize volunteers with the use of a micropipet, or it can be an ancillary activity used during Genome Month.
- One of the best parts of this activity for visitors is ejecting the tip into the disposal cup using the tip ejector button. Be sure to explain the reason behind the button – that scientists do not want to contaminate their samples with substances on their hands and vice versa.

Procedure

If your kit contains a 25 µl fixed volume micropipet, skip directly to step 2.below. Those with an adjustable volume pipet will follow step 1 below:

1. Turn the ring in the middle of the micropipet until the dial reads “25”. This will allow you to pick up a volume of 25 µl with the micropipet. If at any time you would like to change the volume delivered by the pipet, turn the ring to the right

2. or left to increase or decrease the measured volume. Do not attempt to rotate the ring past the limits of the micropipet or you will damage the working mechanisms.
3. Grasp the micropipettor in the palm of your hand and wrap your fingers around the barrel; work the plunger with the thumb. Hold the micropipettor in a vertical position when filling or expelling solution.
4. The digital micropipettors have a two-position plunger with friction "stops". Depressing to the first stop measures the desired volume. Depressing to the second stop introduces an additional volume of air to blow out any solution remaining in the tip. Notice these friction stops; they can be felt with the thumb by pushing on the plunger.
5. *Firmly* place a proper size pipet tip on the end of the micropipet by pushing the tip of the micropipet into the open end of the tip. **Do not use your fingers to place a tip onto the micropipet.** In a laboratory setting, this would contaminate the tip and your sample.
6. Grasp a 1.5 ml microfuge tube, holding the tube between your thumb and forefinger. Open the top of the tube by flipping up the tab with your thumb. During manipulations, grasp the tube body (rather than the lid), to provide greater control and to avoid contamination of the mouth of the tube.
7. When withdrawing or expelling liquid, always hold the microfuge tube firmly between your thumb and forefinger. Hold the tube nearly at eye level to observe the change in fluid level in the pipet tip. Do not pipet with the tube in the test rack. Do not have another person hold the tube while you are pipetting.
8. To withdraw the sample from a reagent tube:

a. Depress the plunger to the *first stop* and hold it in this position. Dip the tip just into the top of the solution to be pipetted, and draw fluid into the tip by *gradually* releasing the plunger. Be sure that the tip remains in the solution while you are releasing the plunger.
b. Slide the pipet tip out along the inside wall of the reagent tube to dislodge any excess droplets adhering to the outside of the tip.
c. Check that there is no air space at the end of the tip or air bubbles with the sample in the tip, carefully expel the sample back into its supply tube.

9. To expel the sample into a reaction tube or into the well in an agarose gel:

a. Touch the tip of the pipet to the inside wall of the reaction tube into which the sample will be emptied. This helps draw the fluid out of the tip.
b. *Slowly* depress the plunger to the first stop to expel the sample. Depress to the second stop to blow out the last bit of fluid. Hold the plunger in the depressed position.
c. Slide the pipet out of the reagent tube with the plunger depressed, to avoid sucking any liquid back into the tip.
d. Eject the tip into a beaker or cup kept on the lab bench by pressing down on the tip-ejector button found on the top of the micropipettor **or**
e. Rinse the pipet tip in a clean cup of water to remove all traces of reagent. Eject the tip back into the box or in the cup used to hold used tips.

Activity Clean-up

1. Wipe the outside of the micropipet with a soft cloth.
2. Replace any used tips in the box or dispose of used tips in the trash.
3. Return micropipettor to the box, store all supplies in the kit.

Gel Electrophoresis

Ever wonder how the banding patterns in DNA paternity or forensic cases are formed? Using a process known as gel electrophoresis, DNA fragments can be separated based on their size and charge.

Gel Electrophoresis At a Glance

Goal	Separate molecules of different sizes using gel electrophoresis
Level	Introductory
Age	6 and above (younger with adult assistance)

Molecules can be separated by size and charge using a method known as agarose gel electrophoresis. It is one of the most common methods used by researchers to isolate and identify fragments of DNA. Agarose forms a matrix that acts as a sieve through which smaller molecules can migrate faster than larger ones. Different matrices can be used to separate DNA molecules that differ by only a single base pair or are chromosomal in size.

To prepare an agarose gel, agarose is heated in a buffer solution and poured into a gel-casting chamber fitted with combs creating a series of sample wells. As the gel cools to room temperature, it becomes a semi-solid matrix (much like Jello) with microscopic holes. Samples are loaded into the wells and an electrical field is applied across the gel. This causes the molecules to migrate through the gel toward one of the electrodes (positive or negative) depending upon their charge. Most proteins and DNA are negatively charged and move toward the positive electrode. Separation of molecules of different sizes can take only a few minutes or a few hours depending upon the agarose concentration and the voltage applied to the gel. Gels containing DNA fragments are typically stained with a DNA sensitive dye such as ethidium bromide or bromophenol blue. The gels can then be documented by photography when illuminated under UV or white light.

The following exercise provides a basic introduction into the theory and practice of gel electrophoresis using samples prepared from food coloring. Idealized results should demonstrate that all complex colors (purple, green, orange) are made up of the primary colors red, blue and yellow. These colors each have a distinctive molecular size that can be separated using electrophoresis. Separation of the colors occurs very quickly (5-10 minutes) and is appropriate for children of all ages. Younger participants may need assistance in handling the micropipet and loading samples but everyone loves the tip ejector!

Equipment and Supplies

50X TAE (Tris-acetate-EDTA).buffer (recipe follows)
Electrophoresis chamber and casting tray, combs
Micropipettor and tips
Power supply
Prepared food coloring samples (recipe follows)
Beaker or waste container for used tips.
Clean container of water if tips are to be rinsed and reused
Agarose

Things you will need that are not supplied as part of this kit

Distilled water
Plastic cups or disposal containers for pipet tips
1 liter container for gel buffer
Microwave oven or heating plate
250 ml flask

Activity Set-up

1. Dilute 50X TAE with distilled water. Using a graduated cylinder, measure out 20 ml of 50X TAE buffer. Dilute to 1 liter with distilled water. Store tightly capped at room temperature.
2. Remove contents of electrophoresis box and place on the table.
3. Unpack the power supply and plug it into an outlet.
4. Prepare agarose gel.
5. Fill the electrophoresis box with 1X TAE buffer to a level that barely covers the entire surface of the gel.

Preparation of the Agarose Gel

Makes 150 ml. Gels can be made ahead of time and stored under damp towels in a sealed container at 4°C.

1. Weigh out 1.2 grams agarose (for a 0.8% gel). This is enough to fill six individual casting trays. Place in a 250 ml flask.
2. Add 150 ml of 1X TAE
3. Melt the agarose using one of the following two methods:
    a. Heat in a microwave on high for one or two minutes, swirl the solution at one minute.
    b. Cover the flask with aluminum foil and heat in a boiling water bath until agarose is melted.

4. Seal the ends of the gel-casting tray with rubber dams, and insert the well-forming comb. Place the gel-casting tray out of the way on the lab bench so that the agarose can set undisturbed.

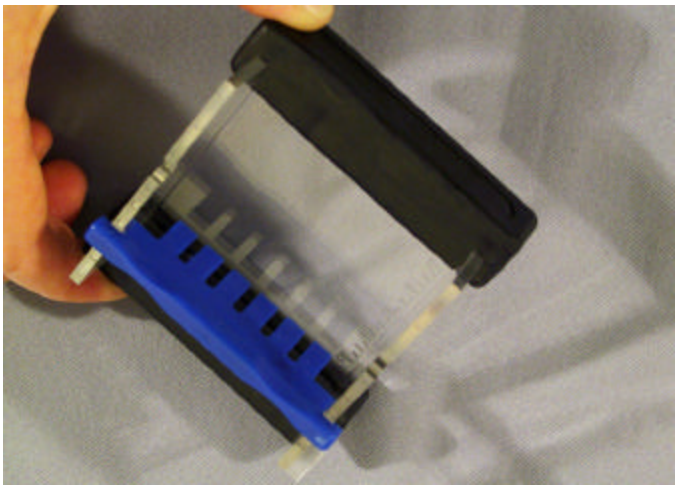

Figure 4. Casting tray with comb and rubber dams in place.

5. Allow the agarose to cool slightly before pouring into the casting tray. Hot agarose can cause the tray to crack.
6. Carefully pour enough agarose solution into the casting tray to fill it to a depth of about one-third the height of the comb teeth. While the gel is still liquid, use a pipet tip to move large bubbles or solid debris to the sides or end of the tray.
7. The gel will become cloudy as it solidifies (in about 10 minutes). *Be careful not to move or jar the casting tray while the agarose is solidifying.* Touch a corner of the agarose *away* from the comb to test whether the gel has solidified.
8. When the agarose has set, unseal the ends of the casting tray. Slide a small metal spatula or the end of a pipet tip between the agarose and the rubber dam to release the seal formed between the dam and the gel.
9. Gently remove the comb, pulling it straight up and out of the set agarose. Do not rock or wiggle the comb.
10. Store the agarose gels in a tightly sealed, refrigerated, humid environment until ready to use.
11. Gels can be loaded either in the electrophoresis chamber with buffer around them or on the bench top. If you load the gels on the bench top, be sure to **fill the wells with buffer beforehand.**
12. Place the casting trays on the platform of the electrophoresis box, so that the wells are at the negative (black) electrode end matching the notches in the chamber.

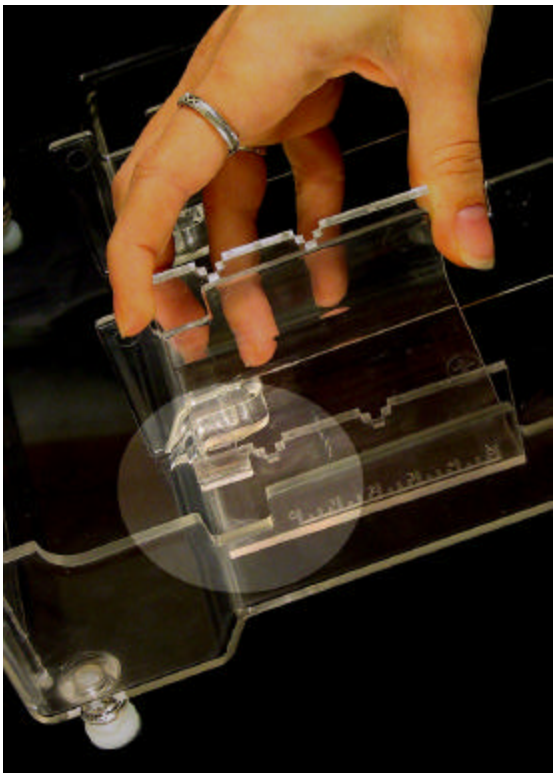

Figure 5. Proper alignment of the casting tray in the electrophoresis chamber

Things to think about when using this activity

- Each casting tray has six sample wells. With the six casting trays this provides opportunities for 36 visitors to run samples at the same time. Once the colored samples reach 2/3 of the way down the gel, another set can be loaded into the wells. The previous samples will run off the bottom of the gel into the buffer solution. This allows many samples to be running continuously.
- You can choose to load samples into the agarose gels either "wet" or "dry". "Wet" is when the casting trays are placed in the electrophoresis chamber and buffer is added just up to and filling the wells. "Dry" is either on the bench top or in the electrophoresis chamber without buffer. If you choose to load your sample using the dry method, be sure to **fill the wells with buffer prior to loading your samples**. Be very careful when placing the casting trays in the electrophoresis buffer or filling the chamber up with buffer or you will lose your samples.
- When loading samples into the wells, the micropipet tip should be positioned in the center of the well just below the surface of the buffer. You do not want visitors poking the end of the tip into the agarose at the bottom of the well. The gels are fragile and pipet tips can dig into the bottom of the well, poking all the way through. It is sometimes helpful if the volunteer or staff personnel help to position the tip in the well by holding onto the shaft of the micropipet. Encourage your visitors to brace their arm on the table top and use their other hand to steady the tip.

- Once a gel is fully loaded and ready to run, it should be placed in the electrophoresis chamber and **gently** surrounded by buffer. The buffer level should come up just to the top of the wells. Be careful when placing the trays in the chamber that you don't plop them down and swish away all of the colored samples.
- It typically takes about 5-10 minutes before the colors begin to separate. You should see bubbles forming on the metal leads once the power is connected. Separation is usually complete in 20-60 minutes.
- This activity does involve the use of electric current at high voltage. Even though there is an interlock remember to caution your visitors when using the equipment.

Procedure

The samples used in this exercise consist of food coloring (a few drops) in a buffer solution with a few drops of glycerin to help the sample sink to the bottom. You can make up your own color combinations, or use the prepared samples.

1. Firmly set a pipet tip onto the end of the 25 µl micropipet.
2. Holding the sample tube in one hand, flip up the top of the tube with your thumb.
3. Push down on the plunger of the micropipettor to the **first** stop and hold it in place while placing the tip into the sample tube. Don't push the tip too far down into the sample solution or the food coloring will adhere to the outside of the tip and leak onto the gel.
4. Release the plunger and remove the micropipettor tip from the sample tube.
5. With your thumb, snap the cap back onto the tube and place the sample tube back into the rack.
6. Load your sample into a clean well slot.
    a. Steady the pipet over the well, using two hands.
    b. If there is air in the end of the tip, carefully depress the plunger to push the sample to the end of the tip. (An air bubble ejected into the well can form a "cap" over the well, causing the DNA/loading dye solution to flow into the buffer around the edges of the well.)
    c. Center the pipet tip over the well, dip the tip in only enough to pierce the buffer surface, and then gently depress the pipet plunger to slowly expel the sample. Take care not to poke the end of the pipet tip into the bottom of the well. The tips are sharp and can poke a hole in the fragile gel.
7. Eject the used tip into the proper waste container or rinse the tip in a cup of clean water. Eject the tip back into the box or a holding container.
8. When all the samples have been loaded into the gel, gently place the tray into the electrophoresis chamber filled with buffer.
9. Place the lid on the electrophoresis chamber, and connect the electrical plugs to the power supply. You should begin to see separation of the colored molecules within 5-8 minutes.
10. Allow the gel to run until all the colors are separated, about 15-20 minutes.
11. Unplug the power supply, disconnect the plugs and remove the cover from the chamber.

12. The gel can be saved for another day or disposed of in regular trash. The TAE buffer can be saved for additional electrophoresis activities.

Activity clean up

1. Unplug power supply. Remove leads from electrophoresis chamber.
2. Carefully pour running buffer into storage container. Buffer can be reused many times before it diminishes in effectiveness. Dispose of used buffer down the drain.
3. Rinse chamber with clean water.
4. Clean out agarose from casting trays. Agarose can be disposed in regular trash. Rinse casting trays with clean water.
5. Dispose of used tips in trash or rinse for reuse.

Questions that may come up about this activity

What does this exercise tell you about the properties of the red, blue and yellow molecules in relation to their size and charge?
All of the molecules are negatively charged, since they migrate toward the positive (red) electrode. From their migration patterns, the yellow molecule travels the furthest, followed by the red and the blue. Therefore, the yellow molecule must be the smallest, followed by red and the blue molecule is the largest of the three.

What would happen if the chamber contained water instead of a buffer solution?
If the chamber contained water, there would be very few ions present to establish an electric field and thus no current produced. The food coloring molecules would not move through the gel and would stay in one place.

How would a higher agarose concentration change the migration of molecules through the gel?
Higher concentrations of agarose (1.0-1.5%) are used to separate smaller fragments of DNA because the pore sizes in the gel matrix are smaller. A higher concentration of agarose would cause the smaller bands to move more slowly through the gel.

Measure the distance each color migrated through the gel. Can you make some estimates on the size of each of these molecules based on the distance traveled?
The size of each molecule is roughly proportional to the inverse $\log_{10}$ of the distance traveled.

Recipes for Buffers and Solutions

50X TAE can be ordered from many different suppliers. The buffer provided with this kit was provided by Edvotek (catalog # 607-1L). If you would like to make up your own buffer solutions, the recipe is given below.

50X TAE (Tris/acetate/EDTA) Electrophoresis buffer
Makes 1 liter. Store at room temperature.

1. Measure ~900ml distilled H_2O.
2. Add 242g Tris base (m.w. 121.1, Sigma catalog #25,285-9)
3. Add 57.1ml Glacial Acetic Acid.
4. Add 18.6 g EDTA (ethylene diamine tetraacetic acid, disodium salt, m.w. 372.24, Sigma catalog #E2,628-2)
5. Adjust volume to 1L with additional distilled H_2O.

Food coloring samples

In a 1.5 ml microfuge tube place one or two drops of food coloring, mixing primary colors to give complex colors. Dilute to 0.5 ml with 1X TBE. Add an equal volume of glycerol (0.5 ml) and mix well.

Sequencing Simulation

Scientists deciphering the human genome now use a standard automated procedure to determine the exact sequence of bases in DNA (estimated to be approximately three billion in humans). This process will be completed two years ahead of schedule. In this activity, visitors can explore the process used to determine a DNA sequence using graphical descriptions of the sequencing procedure and several interactive activities.

Sequencing Simulation At a Glance

Goal	To give visitors an opportunity to understand the process used in sequencing a genome
Level	Introductory to advanced
Age	Middle school visitors and above

DNA sequencing was first developed in 1974 by Frederick Sanger when he was attempting to uncover the amino acids in the insulin protein. At that time, scientists could only sequence a few base pairs of DNA per year and it was very expensive to do. Over the years, technological improvements and automation have increased the speed and lowered the cost of sequencing to the point where individual genes can be sequenced within days and some labs can sequence well over 100 million bases per year. The basic technique discovered by Sanger, however is still in use today.

In sequencing a genome, researchers begin by breaking the chromosomal DNA into large pieces and placing them into bacterial or viral vectors to make what they call libraries of DNA. Like their namesake, DNA libraries store the genetic information in a form that is easy to access and manipulate. These DNA are pieces of around 500-2000 base pairs in size and are inserted into circular pieces of DNA called plasmids. The bacterium carrying the plasmids., typically *E. coli*, is very easy to grow and can produce many copies of the same plasmid.

Sequencing procedures still use the Sanger method of dideoxy chain termination. The dideoxy method gets its name from the role played by artificial nucleotides which lack a hydroxyl group essential for continuing a DNA chain. The dideoxynucleotides can be added to a growing DNA strand, but when added, strand elongation stops because there is no terminal hydroxyl to attach the next nucleotide. An example of a dideoxynucleotide is shown below:

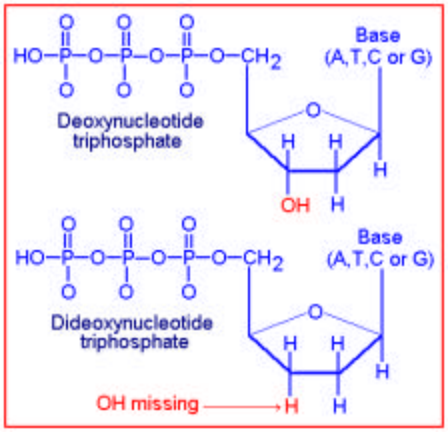

Figure 6. Deoxy and dideoxy nucleotide triphosphates. Graphic courtesy of The University of Reading, Department of Food Science and Technology, *Practical Introduction to Genetic Engineering*, Dr. R.A. Rastall, http://www.fst.rdg.ac.uk/courses/fs761/Topic5/Topic5.htm

The Sanger method creates a set of random copies of the unknown sequence of all different sizes. In the past, sequencing reactions relied on the use of radioactivity (specifically ^{32} P) to visualize the DNA fragments. Today, sequencing reactions incorporate fluorescent labels that can be excited by a laser detector.

To start the sequencing process the following are combined:

All four naturally occurring deoxynucleotides (dATP, dTTP, dGTP, dCTP)
Four fluorescent labeled dideoxynucleotides (ddATP, ddTTP, ddGTP, ddCTP)
Plasmids containing a DNA segment of unknown sequence (called the template)
DNA polymerase (an enzyme that can copy DNA)
A primer (a small sequence of DNA essential for initiation of replication)

The primer is complementary to a short segment within the plasmid DNA. When this short segment of DNA adheres to the plasmid DNA, the polymerase enzyme catalyzes the synthesis of a complementary strand of DNA using the template as a guide. The polymerase continues extending the DNA strand adding nucleotides like building blocks until it randomly incorporates a dideoxynucleotide. The DNA strand can not be extended after a dideoxynucleotide is incorporated because an essential part is missing. When dideoxynucleotides are incorporated, strand synthesis stops. The polymerase then starts over again, synthesizing another DNA strand, beginning at the primer, until another dideoxynucleotide is incorporated. The reaction continues over and over again until the polymerase runs out of steam or ingredients. This process generates thousands of DNA fragments of random sizes. Statistically, strands covering over approximately 800 base pairs of DNA will have been randomly synthesized. This process is illustrated below:

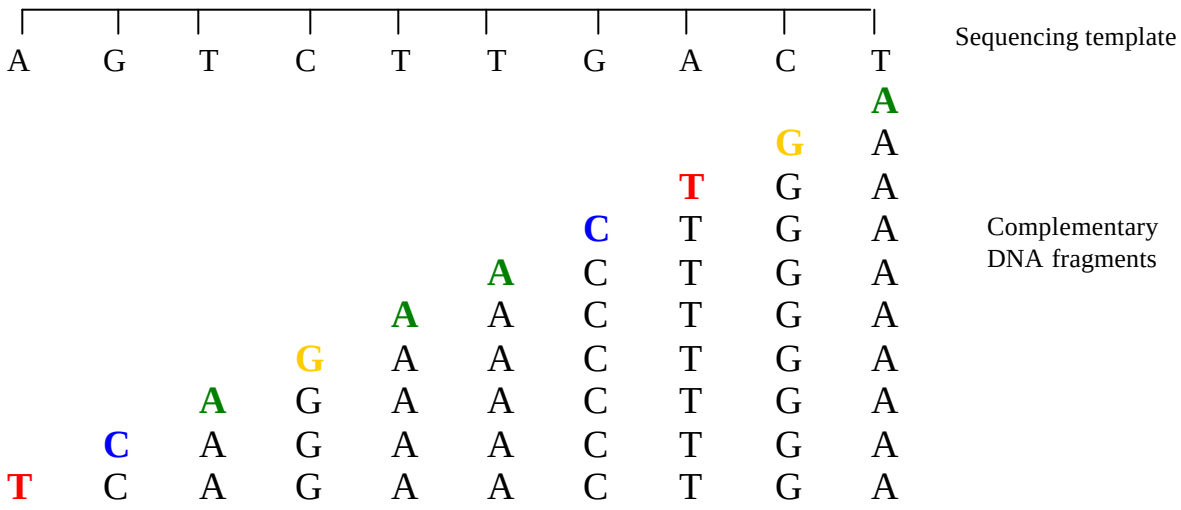

Figure 7. DNA fragments formed during Sanger dideoxy chain termination sequencing

The sequencing reaction is then analyzed by a DNA sequencing machine. These machines separate the DNA fragments using capillary electrophoresis. The capillary arrays are long, thin glass tubes filled with a solution that can separate fragments of DNA differing in size by a single nucleotide. The DNA fragments are pulled through the matrix in the capillary tubes by an electrical current. Small fragments of DNA pass quickly through the tube, while longer fragments travel slower. As each fragment of DNA leaves the capillary tube, a laser excites the fluorescent dye, and the signal is recorded by a computer. These signals make up a chromatogram that is used to determine the unknown DNA sequence. An example of a chromatogram taken from a baboon cystic fibrosis gene is shown below

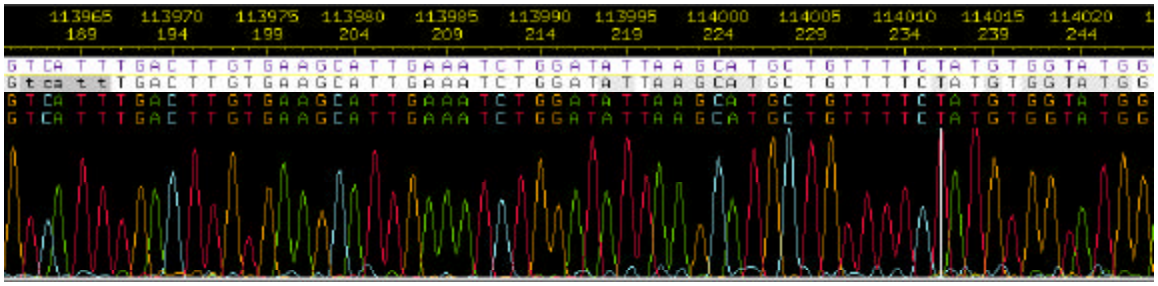

Figure 8. DNA sequence chromatogram from a baboon cystic fibrosis gene

Each peak within the chromatogram corresponds to a DNA fragment differing from the previous by a single nucleotide. The sequence above would be read from left to right (smaller DNA fragment to larger) as GTCATTTGACTTGTGAAGCATT……

This method using fluorescent dideoxy nucleotides has been used to determine the entire sequence of the human genome. In addition, a number of genomes from other species have been sequenced; baker's yeast (*Saccharomyces cerevisiae*), round worm

(*Caenorhabditis elegans*), fruit fly (*Drosophila melanogaster*) and a plant (*Arabidopsis thaliana*). A comparison of these genomes with the human genome has indicated some surprising sequence homologies.

DNA sequencing is a very difficult process to grasp. It is an even harder task to come up with an activity that accurately simulates this process. For these reasons, a variety of different interactive tools have been incorporated into the DNA Kit:

1. A graphic panel outlines the process involved in sequencing an unknown piece of DNA. Visitors can 'walk' through the process in a simplified form.
2. Capillary array units from actual DNA sequencing machines lend an authenticity to the technique.
3. The process of the dideoxynucleotide chain termination can be simulated using pop beads.
4. Simulated sequencing chromatograms can be interpreted by visitors and the results analyzed based on known sequences.

We have tried to include a number of visual and interactive tools in this activity, to aid in helping the visitor understand the process used in determining the sequence of an unknown stretch of DNA.

A. Sequencing Graphics Panel

This graphic panel is a simplified version of the procedure used to sequence a genome. It does not have a lot of detail, but mainly outlines some of the key points involved in sequencing DNA. It may be helpful for staff, volunteers and students to review the tutorial included in the *Human Genome Project Educational Kit*, *Exploring our Molecular Selves*. There is a very good lesson about DNA sequencing on this CD. The tutorial is also available through the NHGRI website at http://www.genome.gov/Pages/EducationKit/online.htm

B. Capillary Array Electrophoresis Unit

This is a disposable unit used by the Human Genome Project Sequencing Centers in the DNA sequencing machines. All of the capillary electrophoresis units included in the kits were donated by either Washington University Genome Sequencing Center in St. Louis, MO or the Whitehead Institute/MIT Center for Genome Research in Cambridge, MA. The units are typically used twenty times before they must be replaced with new units. Some of the larger sequencing centers go through hundreds of these units in a few months. A picture of one of the units in a DNA sequencing machine is located in the upper right hand corner of the sequencing graphics panel.

Each capillary array contains 96 individual glass tubes. These are the gold hair-like threads between the two end pieces. There are two rows of 48 capillary tubes each. Inside each of these capillary tubes is a liquid matrix, capable of separating DNA fragments differing by only a single nucleotide. The prong end of the capillary array is

placed over a 384 well plate holding the sequencing reactions, the other end is attached to the laser detector. The pronged end of the capillary array unit dips down into the solution in the wells. A very small volume, approximately 90 nanoliters (that's 1/1,000,000 or 1 millionth of a ml) is sucked up into the capillary tube. The solution is pulled through the tubes by an electrical current and the DNA fragments begin to separate based on their size. The smaller fragments can migrate through the matrix in the tube much faster than the larger fragments. The larger fragments get hung up and slowed down traveling through the liquid in the tube.

As the fragments emerge from the end of the capillary array, a laser beam excites the fluorescent tag and the color emitted is detected and recorded using a computer. The computer records the elution of each DNA fragment as a peak on a chromatogram. These peaks correspond to a single size of DNA fragment differing from the previous by a single nucleotide. The sequence of the unknown piece of DNA can then be read by stepping across the peaks in the chromatogram.

Each computer is recording the results of 96 different sequencing reactions simultaneously. It takes about eight hours to process the entire plate of 384 sequencing reactions. With an average size of 800 base pairs per sequencing reaction, this results in approximately 300,000 base pairs per eight hour day. Many of the larger sequencing centers run many DNA sequencing machines 24 hours per day, seven days a week. That is a lot of DNA sequence that is being generated! The next generation of DNA sequencing machines may utilize a chip technology that is expected to analyze approximately 7 million bases per day.

C. Pop Beads Sequencing Simulation

The pop beads can be used to simulate the Sanger Method of dideoxynucleotide chain termination. Pop beads that are missing the protruding end are perfect for demonstrating how the DNA strand extensions can be terminated by dideoxynucleotides. The pop beads missing the stem end represent the dideoxynucleotides which are missing a terminal hydroxyl.

Equipment and Supplies

10-15 Purple pop beads
50 White pop beads
5 Red pop beads
5 Blue pop beads
5 Yellow pop beads
5 Green pop beads
10 Red pop beads without the stem
10 Blue pop beads without the stem
10 Yellow pop beads without the stem
10 Green pop beads without the stem
Wavy pop beads
Containers or small bags

Things you will need that are not supplied with this kit

Small containers or plastic bags

Activity Set-up

1. Create a plasmid. Using the purple beads, snap 10-15 purple beads together in a long chain. Add a wavy bead to one end. Attach 10 colored beads in a random manner, trying to have at least two of each color.
2. Remove the stems from the colored beads using a scissors.
3. Place the pop beads missing stems and the white beads into one container.
4. Place the wavy beads into a small container. The wavy beads represent the primers.

Things to think about when using this activity

- The visitors will be synthesizing the complementary strand using the plasmid as a template. In essence, their hands are doing the work that the polymerase enzyme would do.

  Visitors should understand the principle of opposite strand base pairing – a red (T) is always across from a green (A), a blue (C) is always across from a yellow (G). They must recognize the opposite color when synthesizing the complementary strand. For example:

  Whenever a green bead is on the template, they will need to add a red bead to their growing strand.

  Whenever a red bead is on the template, they will need to add a green bead to their growing strand.

  Whenever a blue bead is on the template, they will need to add a yellow bead to their growing strand.

  Whenever a yellow bead is on the template, they will need to add a blue bead to their growing strand
- It may help to have the pop bead DNA model assembled and available to help visitors see the complementary base pairing.
- The visitors will be randomly selecting beads from the container with the colored and white beads. If the color is complementary to the template they can add it to the growing DNA strand. The color beads will terminate the chain because they lack the stem for attaching to the next nucleotide. If the visitor selects a white bead, the chain continues to elongate.
- The wavy bead is the primer. You always start the DNA fragment with a primer.
- The stems should be on the right when synthesizing the DNA chain.

Procedure

1. Give each group a plasmid template, a container holding colored pop beads missing their stems and the white beads and some wavy pop beads.
2. Explain the dideoxy chain termination procedure and that their hands are going to do the work that the polymerase enzyme would normally do. They are going to be making complementary DNA fragments using a completely random process.

3. Make sure the visitor understands that they will be making the complementary (opposite) strand using the white and colored beads. The base pairing should follow the rule:

   RED (T):GREEN (A)
   BLUE (C):YELLOW (G)

4. Have each group start by placing a wavy bead (primer) under the wavy bead (complementary primer sequence) on the template. The stem should be pointing to the right.
5. Begin building the DNA chain by randomly selecting beads from the container. If a white bead is selected, add that to the growing DNA chain and go to the next bead on the template. If a colored bead is selected, check to see if it is complementary to the template. If not, select another bead.
6. When the complementary color is added to the DNA strand, the chain terminates because the colored beads are missing their stems. Another bead (nucleotide) can not be added to the chain.
7. Begin building a new DNA chain starting with a primer. Continue building complementary DNA sequences until you have used up all of the beads, or have ten different fragments each differing from the previous by a single nucleotide.
8. If you have more than ten white beads in a row, take them off and start again.
9. Arrange the fragments from smallest (single nucleotide) to largest (ten nucleotides).
10. "Read" the DNA sequence using the colored beads on the end of each chain as your code. Those fragments with a green beads would be an "A", red would be "T", blue is "C" and yellow is "G".
11. This process imitates the procedure that is used in the capillary array to separate fragments of different sizes.

Activity clean-up

1. Take apart all of the beads, separating them into their containers.
2. Save the plasmids for the next group

D. Sequencing Scans

Equipment and Supplies

Sequencing traces (eight different traces)
DNA sequences for known diseases and traits
Washable markers

Things you will need that are not supplied with this kit

Paper towels or tissues

The sequencing scans are a fun way for visitors to practice interpreting and analyzing the data from a sequencing reaction. Using washable markers, visitors record the sequence in the shaded box above the tracess. Each peak corresponds to a single DNA fragment, differing from the previous by a single nucleotide. The sequence can be read from left to right, smallest DNA fragment to largest.

Once the sequence is recorded the data can be compared to known DNA sequences for selected diseases and traits. Visitors match their recorded sequence with these known sequence to decode their unknown gene.

The recorded sequence can easily be erased using the paper towels.

Sequence Analysis

What kind of information is contained within a genetic sequence? In this activity visitors can analyze the sequence for the genetic disease, cystic fibrosis. Unraveling the sequence has lead scientists to a deeper understanding of the genetic basis for this disease and hopefully will eventually lead to a cure.

Sequence Analysis At a glance

Goal	To give visitors a look into the details of single gene, coding for cystic fibrosis
Level	Introductory
Age	Visitors of all ages; younger children may have difficulty with some of the terminology

Cystic fibrosis (CF) is the most common fatal genetic disease in the United States today. It is estimated that over 30,000 individuals are inflicted with this disease nationwide and that 1,000 new cases are diagnosed each year.[3] Despite major milestones in the research and management of this disease, CF remains a challenging and incurable health problem.

Cystic fibrosis is a recessive genetic disease characterized by improper functioning of the epithelial cells lining the lungs and other organs. The ducts contained within the tissues lining the pancreas, intestines, reproductive system and lungs become clogged due to the formation of a thick, sticky mucus. This leads to difficulty in digestion and absorption of food in the pancreas and intestines, blockages of the bronchial airways and infections in the lungs, and infertility, especially in men.

In 1989, researchers identified the gene responsible for cystic fibrosis.[4,5] It is located on chromosome 7 and was named the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is a protein involved in transporting chloride molecules across the membrane of epithelial cells. You can think of this protein as a gatekeeper that maintains a balance of sodium and chloride ions between the inside and the outside of a cell.

In normal lung cells, a balance exists between sodium, chloride and water. The mucus is smooth and wet and can trap bacteria and other debris. Tiny hair-like projections called cilia beat back and forth, moving the mucus and trapped particles out of the lungs and up to the throat where they are coughed out. In patients with cystic fibrosis, the cells are

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[3] M. J. Welsh and A. E. Smith. "Cystic Fibrosis." *Scientific American* **273** (6): 52-59 (1995)

[4] J. M. Rommens, M. C. Iannuzzi, B. Kerem, M. L. Drumm, G. Melmer, M. Dean, R. Rozmahel, J. L. Cole, D. Kennedy, N. Hidaka, M. Zsiga, M. Buchwald, J. R. Riordan, L. C. Tsui, and F. S. Collins. "Identification of the Cystic Fibrosis Gene: Chromosome Walking and Jumping." *Science* **245** (4922): 1059-65 (Sept. 8, 1989).

[5] J. R. Riordan, J. M. Rommens, B. Kerem, N. Alon, R. Rozmahel, Z. Grzelczak, J. Zielenski, S. Lok, N. Plavsic, J. L. Chou, M. L. Drumm, M. C. Iannuzzi, F. S. Collins, and L. C. Tsui. "Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA." *Science* **245** (4922): 1066-73 (Sept. 8, 1989).

missing or have a defective CFTR protein. This prevents chloride from being released from the cells resulting in a salt imbalance. Sodium and water move back into the cell causing the mucus to become thick and dry. The thick mucus traps bacteria and is difficult for cilia to move. The bacteria begin to multiply in the mucus, setting up an infection. White blood cells come to fight the infection as part of the immune response and they themselves die. The long stringy strands of DNA from all of these ruptured cells causes additional blockage and damage to the lung tissue. Patients with cystic fibrosis suffer repeated bouts of infection, leading to scarring and damage to the lungs.

In the past decade, researchers have identified the defect in the DNA that causes 70% of the cystic fibrosis cases.[6] Francis Collins, Director of the National Human Genome Research Institute (NHGRI) at the National Institutes of Health (NIH), was one of the researchers who found that a deletion of just three nucleotides (CTT) and a single amino acid (phenylalanine) in the CF gene leads to the complications of the disease. This deletion caused the CFTR protein to either be missing from the surface of affected cells or to be ineffective in regulating chloride transport.

Recently, scientists have been working on developing a treatment strategy involving gene therapy for those patients suffering from cystic fibrosis. Attenuated virus particles or other carriers are used to transport the normal CFTR gene to affected lung tissues. Although the addition of the normal gene to CF cells is successful in cultured cells in the laboratory, clinical results have been less successful than originally hoped. Much more work will need to be accomplished before adequate therapies for the treatment of cystic fibrosis are available.

Recently, another strategy for the treatment of cystic fibrosis has been initiated by investigators at the University of Washington's Genome Center and at PathoGenesis Corporation. Researchers there have recently completed sequencing of the genome of the bacterium *Pseudomonas aeruginosa,* thought to be the primary causative agent of lung infections in cystic fibrosis patients. It is hoped that the information gained by sequencing this bacterial genome will lead to the design of drugs which will be effective in treating cystic fibrosis.

Cystic fibrosis is a recessive autosomal trait, which means that individuals need two copies of the defective gene in order to express the disease. Children will only inherit the disease when both parents are carriers, or when one parent has cystic fibrosis and the other parent is a carrier. As presented in the Punnett square below, when both parents are carriers of the disease, they have a 25% (1 out of 4) chance of passing the trait onto their children. Those individuals who do not carry a normal copy of the CFTR gene (F) will have the cystic fibrosis disease.

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[6] B. Kerem, J. M. Rommens, J. A. Buchanan, D. Markiewicz, T. K. Cox, A. Chakravarti, M. Buchwald, and L.C. Tsui. "Identification of the Cystic Fibrosis Gene: Genetic Analysis." *Science* **245** (4922): 1073-80 (1989 Sep 8).

Parental genotype

	F	f
F	FF	Ff
f	Ff	ff

Offspring phenotype

25 % FF – homozygous allele for normal CFTR gene
50% Ff – heterozygous carrier for defective CFTR gene
25% ff – homozygous allele for defective CFTR gene

Cystic fibrosis is most commonly diagnosed by a "sweat test." One of the most common side effects of cystic fibrosis is very salty tasting skin. In normal individuals, sweat produced at the base of the sweat glands is rich in both sodium and chloride. As sweat passes through the gland, the sodium and chloride ions are reabsorbed into the cells, and the sweat released on the surface of the skin is only slightly salty. In patients with cystic fibrosis, the transport of chloride ions is impaired resulting in the release of sweat that is very salty. In ancient times, northern European folklore held that "woe to the child which when kissed on the forehead tastes salty. He is bewitched and soon must die."

In this activity, visitors have an opportunity to view both the genomic and protein coding regions of the CFTR gene. This information was gathered over the course of many years and continues to be an area of intense research interest. Visitors can "read" the gene sequence (all 471,745 bases) and locate the three nucleotides which when deleted cause the fatal genetic disease cystic fibrosis. Visitors can get a sense of the vastness of the genome, since a single gene of average size covers a distance of over forty feet. The magnitude of deciphering the human genome and mining the information contained within is apparent when analyzing the sequence information for this single gene.

A. Cystic Fibrosis Gene Sequence

This is the entire genetic sequence for the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). It is a small piece (471,745 nucleotides) of chromosome seven which is thought to contain approximately 171 million bases. The genomic sequence of CFTR contains 59 regions used to construct the transcript for the CFTR protein. These are commonly called exons and are highlighted in blue. DNA contains a lot of "extra" DNA that is not used when making proteins. This extra DNA is made up of introns and is referred to as "junk DNA." No one quite understands what the function of this extra DNA is. It may be present to minimize the chances that a mutation would occur in a critical section of DNA, or it may be involved in gene control or regulation. Human DNA seems to have more extra DNA than many other species. It is hoped that the completion of the Human Genome Project will help to answer puzzling questions such as this.

The "extra" pieces of DNA are removed when it is copied into RNA (a process called transcription). The extra pieces of RNA (called introns) are cut out and the coding sequences (called exons) are spliced together. The final product after this processing is used to make the protein product. This process is diagrammed in Figure 9:

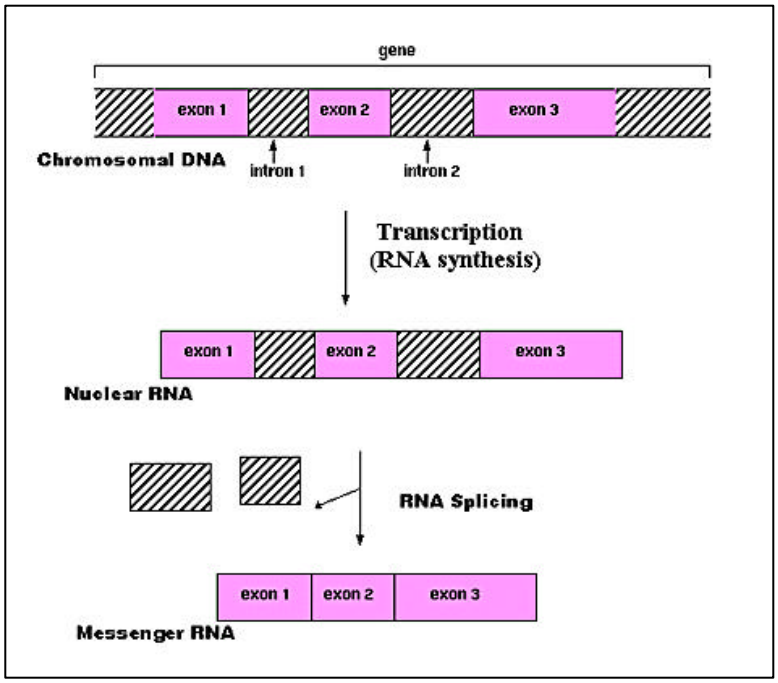


Figure 9. RNA synthesis and processing. Reprinted from Access Excellence Graphics Gallery. http://www.accessexcellence.org/AB/GG/rna_synth.html

The cystic fibrosis sequence contains fifty-nine exons that are spliced together to produce the final RNA transcript. In mammalian cells only about 1% of the DNA is ever used to make a functional RNA product. The purpose of the rest of the sequence is still unknown. All of the RNA processing takes place before leaving the nucleus.

Some interesting facts about the CFTR genomic sequence

- The CFTR gene is found in region q31-q32 on the long (q) arm of human chromosome 7.
- It contains 471,745 nucleotides or bases and 59 exons (highlighted in blue). Twenty seven of these exons (13-39) are translated into protein. The other exons may contain regulatory elements, promotor regions or other important information for gene control.
- The deletion of three nucleotides (CTT) from exon 23 is referred to as the delta F508 mutation (highlighted in red). Approximately 70% of cystic fibrosis cases are a result of this mutation. A delta F508 mutation results in a protein which becomes folded improperly and cannot function as a chloride channel regulator.
- There are approximately 900 different types of mutations, some of which may result in milder forms of the disease.
- Adding a normal CFTR to respiratory membranes through nose sprays or inhalants seems to stimulate production of normal CFTR chloride channel

proteins. Unfortunately, lung epithelial cells turn over fairly quickly, so the effect is short-lived.

- Approximately 1 in 31 Americans are carriers of the cystic fibrosis gene. These individuals do not express any symptoms of the disease but can pass the disease on to their offspring. Carriers can be tested using a simple blood test that detects the most common form of cystic fibrosis. Other mutations will not be detected using this blood test.

B. CFTR Protein coding sequence

The cystic fibrosis transmembrane conductance regulator (CFTR) protein is a chloride channel protein found in cells that form the lining of the pancreas, lungs, colon and reproductive system. The protein is embedded in the membrane of the cells and acts as a gate for the transport of molecules, particularly chloride through the membrane. A schematic drawing of the CFTR protein is presented in Figure 10.

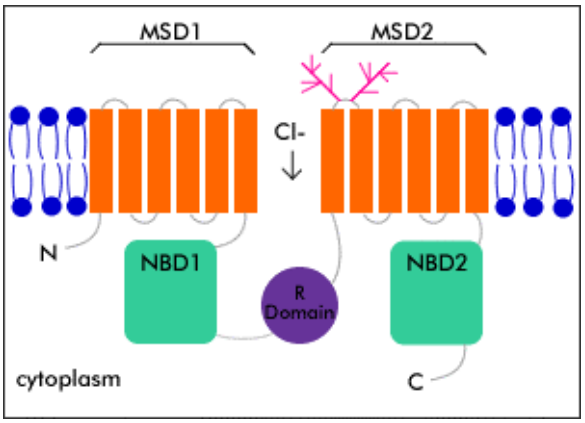


Figure 10. Schematic of the CFTR protein. Reprinted from Gene Gateway-Exploring Genes and Genetic Disorders, U.S. Department of Energy/Biological and Environmental Research Program http://www.ornl.gov/hgmis/posters/chromosome/cftr.html

There are five domains in the CFTR protein: two are membrane spanning regions that make up the chloride channel (MSD1, MSD2, highlighted in yellow), two are nucleotide binding regions that bind ATP [adenosine triphosphate] (NBD1, NBD2, highlighted in pink) and a regulatory domain (R, highlighted in blue). The nucleotide binding domains and the regulatory domain act as gatekeepers for the chloride channel. The delta F508 mutation that causes cystic fibrosis is found in the first nucleotide binding domain.

In many cystic fibrosis cases, the defective protein is made in the cells but never reaches the cell membrane. Processing of the faulty protein is stopped when the cell's quality control mechanisms recognize that the protein is folded incorrectly. Instead of tagging the protein for transport to the membrane, the defective CFTR is marked for degradation.

Some interesting facts about the CFTR protein sequence

- The CFTR protein coding region is 6100 base pairs long and codes for a protein containing 1480 amino acids, with a molecular weight of 168,138 Daltons.
- CFTR is a member of a large class of proteins called an ABC (ATP binding cassette) transporter or Traffic ATPase. These proteins are involved in transporting molecules across membranes. CFTR is unlike other ABC transporters because it contains a regulatory domain. Most ABC transporters only contain four domains: two membrane spanning regions and two nucleotide binding domains.
- The nucleotide binding domains of CFTR do not bind ATP to produce energy as would be needed if CFTR were a pump. Rather the ATP binding domains and the regulatory domains act as a gatekeeper. For unknown reasons, the CFTR protein has developed a sophisticated level of regulation unlike that seen in other species. It relies on degrees of phosphorylation (supposedly from ATP) which opens or closes the "gate" for chloride transport.

Websites that you can visit for more information

http://www.yourgenesyourhealth.org/ygyh/mason/index Dolan Learning Center, Cold Spring Harbor. A multimedia guide for learning about genetic disorders.

http://www.yourgenesyourhealth.org/ygyh/mason/index Gene Gateway- Exploring Genes and Genetic Disorders, U.S. Department of Energy, Biological and Environmental Research Program

http://www.genome.gov/page.cfm?pageID=10001213 Learning about Cystic Fibrosis, National Human Genome Research Institute, National Institutes of Health

http://www.mayo.edu/research/profiles/topic_1044.html Dr. John Riordan, Mayo Clinic, Scientist Profile

http://www.genome.gov/page.cfm?pageID=10000351 Dr. Francis Collins, National Human Genome Research Institute, National Institutes of Health, Scientist Profile

http://www.science.ca/scientists/scientistprofile.php?pID=19 Dr. Lap-Chee Tsui, The Hospital for Sick Children, Toronto, Scientist Profile

Genetic Traits

Half of a person's genome comes from their mother and half comes from their father. Therefore, every person has two copies of each gene for each trait. However, each gene is not always expressed. The following activities allow visitors to explore the topic of dominant and recessive genes, including how they are passed on, how they interact, and some examples of each.

Goal	To give visitors an opportunity to explore inheritance of genetic traits.
Level	Introductory
Age	Visitors of all ages

It was Gregor Mendel's experiments in the mid-1800s with pea plants that first illuminated the nature of dominant and recessive traits. He observed that certain traits were dominant over others and that some hidden traits would show up if the pea plants were crossed in just the right combination. A dominant trait is one that only needs one copy of a gene to be expressed. A recessive trait needs both copies of the gene to be expressed. A parent can pass on a recessive trait to a child without it ever being apparent that the parent has that gene. For example, in some instances, a person with brown hair may have two dominant genes, BB, or one dominant and one recessive, Bb. A person with blond hair may have two recessive genes, bb. What happens if a dark haired person who carries the recessive blond gene, Bb, has children with a blond person, bb? It is easy to figure what color hair their children might have by using a Punnett square:

	B	b
b	Bb	bb
b	Bb	bb

On average, this couple has a 50/50 chance of having either a brown haired (Bb) or blond haired (bb) child. The Punnett square is useful because it allows a comparison of genotypes versus phenotypes. A **genotype** refers to all the genes that a person has. The **phenotype** is the expression of those genes. So in the example above, the brown haired person has a phenotype of brown hair, but a genotype of brown and blond.

The model presented above for predicting brown-blonde hair color is an over-simplification of hair color inheritance. Many individuals do not fit into this model, because there are several different alleles for hair color which have a specific hierarchy for dominance. Some red alleles are dominant to brown alleles, and true black hair is actually recessive, but allows for darker shading which effects brown hair shades. Also, many environmental factors affect hair color such as hair care products, UV light, disease and skin conditions, etc. The hair color model is simply a classical example of Mendelian genetics.

The What's in my Genes? activity is a fun way to look at some of the traits that are easy to define as dominant or recessive. Keep in mind that some traits are controlled by more than one pair of genes. For example, several pairs of genes control eye color. However, one pair plays the largest role in defining the general shade of color, so it is still possible to say which color is dominant or recessive.

Another trait that is controlled by several pairs of genes is the PTC trait. This refers to the ability to taste the chemical phenylthiocarbamide (also called phenylthiourea). In general, 7 out of 10 people can taste the bitter PTC taste, although this number can vary in different populations around the world. The ability to taste PTC is a dominant trait. However, several factors can affect exactly how well someone can taste it, such as the number of taste buds that they have, or how old they are (we tend to somewhat lose our sense of taste as we age), or the concentration of the PTC. Testing for this trait is easy. Remove one piece of PTC-impregnated paper from the pack. Place it on the tongue for several moments. Tasters will almost immediately taste a rather unpleasant, bitter flavor. Non-tasters won't notice anything strange about the paper at all.

Visitors can do the phenotype activity with one another, or you can include a mirror for people to use on their own to determine some of their traits, such as the widow's peak. Definitely have a volunteer on hand if you include the PTC paper in the activity. This will help minimize waste of the small slips of paper and ensure proper disposal of used pieces. Also, visitors might want more information about the PTC trait (refer to the information sheet that comes with the PTC packets). Feel free to make copies of the small, two-sided lists if this is something your visitors would like to check-off and take home with them.

Equipment and Supplies

Copies of phenotype list
PTC paper
Copies of take-home phenotype lists

Things you will need that are not supplied as part of this kit

A table top stand for the phenotype list
Mirror (optional)
Disposal container for used PTC paper
Pencils to use with take-home lists

A. What's in my Genes?

Many of your characteristics are determined by genes, sections of DNA that code for proteins. Each characteristic, or trait, is controlled by a pair of genes, or sometimes more than one pair. A dominant trait is one that only needs one copy of a gene to be expressed. A recessive trait needs both copies of the gene to be expressed. For example, a person with brown hair can have two dominant genes, BB, or one dominant and one recessive, Bb. A person with blond hair will have two recessive genes, bb. Some traits, such as eye color, are determined by several sets of genes. With eye color, one set of genes plays the biggest role in determining whether the color will be light or dark.

Figure out which of your traits are dominant and which are recessive. Use a pencil to record your observations. You might need to do this with a friend or use a mirror.

Visible Trait (phenotype)	Dominant	Recessive
1. Ear Lobes	☐ free	☐ attached
2. Hair Type	☐ curly	☐ straight
3. Tongue rolling	☐ can roll	☐ cannot roll
4. Hair on middle joint of fingers	☐ hair present	☐ hair absent
5. Widows Peak	☐ present	☐ absent
6. Freckles	☐ present	☐ absent
7. Eye color	☐ brown, hazel, green	☐ blue, gray
8. Hitchhiker's thumb	☐ curved	☐ straight
9. Hair color	☐ black, brown	☐ blond, red
10. Dimples	☐ present	☐ absent
11. Eye lashes	☐ long	☐ short
12. Chin cleft	☐ present	☐ absent
13. Handedness	☐ right	☐ left
14. PTC tasting	☐ can taste	☐ cannot taste

B. Genetic Roulette

This is very simple activity which gives visitors an opportunity to explore the statistical probability of inheriting a particular genetic trait.

Equipment and Supplies

Poker chips with dominant or recessive markers on them
Plastic cup

Procedure and Activity Set-up

2. Decide which genetic traits you will be demonstrating. Each scenario requires that two poker chips be used, representing the genes donated by the mother and the father.

   Recessive traits and disorders (bb) such as cystic fibrosis require either:
   both carrier genotypes – Bb, Bb
   or one carrier and one affected individual – Bb, bb

   Dominant traits (Bb or BB) require either:
   one carrier and one recessive – Bb, bb
   two carriers – Bb, Bb
   two dominants – BB, BB
   one carrier and one dominant – Bb, BB
   one dominant and one recessive – BB, bb

2. Remove those poker chips that will be needed to play the game. Place the other chips back into the bag.
3. Use the cup to roll the chips. Statistically each roll should give the probability of inheriting the genetic disorder or traits given a certain combination of genes. Example: there is a 1 out of 4 chance (25%) of inheriting a recessive gene from two parents who are carriers of the recessive trait.
4. Use Punnett squares to reinforce the genetic distribution of inherited genes.

C. Herring Sperm DNA

This small vial is filled with a sample of DNA isolated from Herring sperm. This is a rich source of DNA that is easily extracted and purified. It has been dried, preserving its long, fibrous nature. The DNA sample can be used as a prop to initiate conversations about the structure, function and historical aspects of DNA. Our thanks to Tom Zinnen at BioTrek, a Biotechnology Outreach Program at the University of Wisconsin – Madison for this activity. For more information visit his website at:
http://www.biotech.wisc.edu/Education/index.html

Additional Content Material

A. Landmarks of the Human Genome

Human Genome Landmarks wall poster depicts the 24 human chromosomes and some of the genes associated with particular disorders and traits. When talking to visitors about this poster, remind them that we all have copies of these genes, yet most of us never have the trait or disorder expressed. You can order more free copies of the poster by visiting the Department of Energy website "Exploring Genes and Genetic Diseases": http://www.ornl.gov/hgmis/posters/chromosome/

Some common diseases and genetic traits

Disorder or trait	Chromosome	Disorder or trait	Chromosome
Albinism	9	Graves disease	14
Alzheimer disease	1, 14	Green eye color	19
Amy tropic lateral sclerosis	21, 22	Hearing Loss	13
Asthma	5	Hemophilia	X
Atherosclersosis	19	Huntington disease	4
Autism susceptibility	7	Hypertension	3
Baldness	5	Non-Hodgkins lymphoma	1
Blue eye color	19	Obesity	7
Breast cancer	13, 17	Marfan syndrome	14
Brown eye color	15	Paget disease of bone	18
Brown hair color	15, 19	Parkinson disease	4
Carpal tunnel syndrome	18	Psoriasis	1
Cataracts	1	Red Hair	2,4
Colon cancer	2,3	Schizophrenia	5
Creutzfeldt-Jakob disease	20	Sickle cell anemia	11
Cystic Fibrosis	7	Tay-Sachs disease	15
Duchenne Muscular Dystrophy	X	Thalassemia	11,16
Dyslexia	2, 15		

B. The Human Genome Project Educational Kit

"Exploring Our Molecular Selves" multimedia educational kit.

The Human Genome Project (HGP) began in 1990 as an effort by researchers from around the world to map and sequence the human genome - the totality of human DNA - as well as the genomes of important experimental organisms, like yeast, the nematode worm, and mouse. In 2000, the collaborators in the HGP announced the completion of a draft revealing 90% of the human sequence and in February 2001, the initial analysis of the human genome sequence was published in the scientific literature.

To mark this occasion, the Human Genome Project released a free, multimedia educational kit for high school students and the interested public. A limited number of kits were produced and distributed within the first year of the release in February 2001. A copy of this educational kit has been included in your DNA Program Kit. The contents of the education kit were reformatted and are also available on the NHGRI website in two formats; download modules or online viewing at: We recommend that you use this educational kit as part of the staff and volunteer training session and if possible make it available for visitors to use.

http://www.genome.gov/Pages/EducationKit/

The following modules are presented as part of this kit:

Milestones in Genetics: An interactive timeline presenting more than 90 key events in the history of genetics occurring over nearly 150 years. A short, illustrated story describes each key event. In addition, primary references, archival images, and many original scientific publications are available for more detailed study.

Genes, Variation, and Human History: Two interactive classroom activities, *Genetic Variation in Populations* and *Using Genes to Trace Human History* allow students to study the similarities and differences among individuals and among populations.

How to Sequence a Genome: An animated, narrated segment on How to Sequence a Genome presents the essential steps in sequencing an organism's genome.

Ethical, Legal, and Social Implications: A segment on Ethical, Legal, and Social Issues that begins with a video that introduces key themes. Seven case studies are presented with discussion questions to spur dialogue on these important issues.

Glossary of Genetic Terms: A link to the talking Glossary of Genetic Terms presenting written definitions, phonetic spelling, illustrations, and audio of scientists defining scientific terms.

"Exploring Our Molecular Selves": A 3D computer-animated video illustrating the basic components and principles of molecular biology. The video gives a sense of the scale of cells, chromosomes, and DNA and shows how the information in DNA is converted into the molecules necessary for life.

The Secrets of Our Lives: A 15-minute video documentary, *The Secrets of Our Lives,* was awarded a gold medal prize in the New York Film Festival. It weaves together conversations with HGP leaders to trace the development of the project and to address its scientific and societal impact. A montage of film footage, eye-catching graphics, and creative camera angles sustain viewer interest. This video documentary is available in open-caption, English, and Spanish.

The Future of Research and Medicine: Two written essays on The Future of Research and Medicine, which highlight major research areas spurred by genomics and the likely future effects of new genetic knowledge and technologies on medicine.

Genetics: The Future of Medicine: An informational brochure, *Genetics: The Future of Medicine*, details the goals of the HGP and explains its potential consequences. It includes illustrated explanations of basic genetic terms and concepts.

C. Other Resources on the NHGRI web site

Information regarding the American Society of Human Genetics Mentorship Program, a network of genetics/genomics professionals available for outreach activities in your area.
http://www.genome.gov/Pages/EducationKit/mentor.html

Classroom lesson plans designed to accompany the NHGRI multimedia education kit.
http://www.genome.gov/page.cfm?pageID=10005911

Appendix I

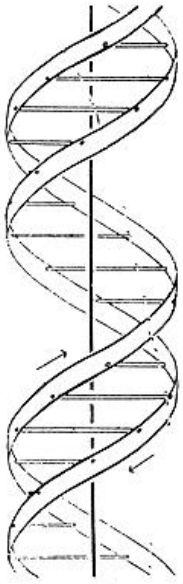
Original publication by Watson and Crick

Nature, Vol. 171, page737, 25 April 1953

MOLECULAR STRUCTURE OF NUCLEIC ACIDS
A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey (1). They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining ß-D-deoxyribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right- handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's (2) model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each every 3.4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. The are joined together in pairs, a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows : purine position 1 to pyrimidine position 1 ; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are : adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine ; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally (3,4) that the ratio of the amounts of adenine to thymine, and the ration of guanine to cytosine, are always bery close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data (5,6) on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON F. H. C. CRICK

Medical Research Council Unit for the Study of Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 25.

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Appendix II

List of suppliers

Carolina Biological
2700 York Road
Burlington, NC 27215
800-334-5551

CDP Industries
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800-621-7193

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www.edvotek.com

Fisher Scientific
711 Forbes Ave
Pittsburgh, PA 15219
800-766-7000

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Coon Rapids, MN 55433-5813
612-786-0880
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Fisher Scientific


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Pfizer