ABG706 ADVANCE ANIMAL BIOTECHNOLOGY

BY

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What is Biotechnology?

The term "biotechnology" refers to the use of living organisms or their products to modify human health and the human environment.

The term "biotechnology" was coined in 1919 by Karl Ereky, an Hungarian engineer. Biotechnology described biotechnology as the technique that allow DNA to be manipulated, i.e. to move genes from one organism to another on the one hand or the technique that involves relatively new technologies whose consequences are untested and should be met with caution. for example in the use of stem cells, gene therapy and genetically modified organisms.

EARLY APPLICATIONS AND SPECULATIONS FROM 6000 BC – 1700 AD:

6000 BC Yeast was used to make beer by Sumerians and Babylonians.

4000 BC The Egyptians discovered how to bake leavened bread using yeast.

Other fermentation processes were established in the ancient world notably in China.

Molds were used to produce cheese, vinegar and wine were manufactured by fermentation. The fermentation of milk by lactic acid bacteria resulted in yogurt.

400 BC Hippocrates determined that male contribution to a child's heredity is carried in the semen. By analogy, he guessed there is a similar fluid in women. Since children receive traits from each in equal proportion.

320 BC Aristotle rejected the theory of Hippocrates, said that all inheritance comes from the father's semen, while the mother merely provides the material from which the baby is made. He suggested that female babies are caused by "interference" from the mother's blood.

1100-1700 AD Theory of spontaneous generation, i.e., organisms arise from non-living matter was proposed.

1665 AD Robert Hooke observed the cellular structure for the first time.

1673 AD Anton van Leeuwenhoek used his microscopes to make discoveries in microbiology.

FROM 1700 TO 1900

1701 Giacomo Pylarini in Constantinople practiced "inoculation".

1798 Edward Jenner published his book comparing vaccination (infecting humans with cowpox to induce resistance to smallpox) to inoculation (infecting humans with a putatively mild strain of smallpox to induce resistance to serve strain of the same).

1799 Lazaro Spallanzani described ingeniously crafted experiments to test the possibility of using heat to kill all the microbes in an "infusion".

1809 Nicolas Appert devised a technique using heat to can and sterilize food.

1850 Ignza Semmelweis used epidemiological observations to propose the hypothesis that childbed fever can be spread from mother to mother by physicians.

1856 Louis Pasteur proved that fermentation is the result of yeast and bacterial activity.

1859 Charles Darwin hypothesized that animal populations adapt their forms over time to best exploit the environment, a process he referred to as "natural selection". He emphasized on his idea of "survival of the fittest". His landmark book, "On the Origin of species", was published in London.

1863 Louis Pasteur invented the process of pasteurization, heating wine sufficiently to inactivate microbes, while at the same time not ruining the flavour of the wine.

1865 Gregor Mendel presented his laws of heredity to the Natural Science Society in Brunn, Austria.

1870 W. Flemming discovered mitosis.

1871 DNA was isolated from the sperm of trout found in the Rhine River.

1873-6 Robert Koch investigated anthrax and developed techniques to view, grow and stain organisms.

1878 Joseph Lister described the “most probable number” technique, the first method for the isolation of pure cultures of bacteria.

1880 Pasteur published his work on “attenuated” strains.

1881 Pasteur used attenuation to develop vaccines against the bacterial pathogens of fowl cholera and anthrax.

1882 Walther Flemming reported his discovery of chromosomes.

1884 Pasteur developed a rabies vaccine.

1892 Ivanovsky reported that the causative agent of tobacco mosaic disease is transmissible, and can pass through filters that trap the smallest bacteria. Such agents are called “viruses”

1896 Wilhelm Kolle developed cholera and typhoid vaccines.

1897 Edward Buchner demonstrated that fermentation can occur with an extract of yeast in the absence of intact yeast cells.

Ronald Ross discovered Plasmodium (the protozoan that causes malaria) in the female Anopheles mosquito and showed the mosquito transmits the disease agent from one person to another.

1900 Walter Reed established that yellow fever is transmitted by mosquitoes, the first time a human disease was shown to be caused by a virus.

THE ADVENT OF DNA TECHNOLOGIES: 1900 TO 1953

1900	Mendel's work was rediscovered by three scientists – Hugo de Vries, Erich Von Tschermak, and Carl Correns.
	William Sutton observed homologous pairs of chromosomes in grasshopper cells.
1904	William Bateson introduced the concept now known as ' gene linkage' and 'genetic maps' that describe the order of the linked genes.
1907	Thomas Hunt Morgan in his work with fruit flies proved that chromosomes have a definite function in heredity, established mutation theory that led to the fundamental understanding of the mechanisms of heredity.
1908	BCG vaccine against TB was developed.
	A.E. Garrod described "inborn errors of metabolism"
1928	Fredrick Griffiths noticed that a rough type of bacterium "r strain" changed to a smooth type "s strain" and discovered Transformation.
1935	Stanley crystallized Tomato Mosaic Virus(TMV).
1936	Stanley isolated nucleic acids from TMV.
1939	Gautheret cultivated carrot (callus cultivars).
1940-1945	Large scale production of penicillin was achieved.
1941	"One gene on enzyme" hypothesis by Beadle and Tatum.
1944	Avery, McCarty and MacLeod determined that DNA is the hereditary material inherited in transformation.
1951	Esther M. Lederberg discovered lambda phage.
1952	Zinder and Heidelberg discovered Transduction process.
1953	Watson and Crick proposed the double stranded, helical complementary, anti-parallel model for DNA.

EXPANDING THE BOUNDARIES OF DNA RESEARCH: 1953-1976

1953 Gey developed the HeLa human cell line.

1957 Francis Crick and George Gamov worked out the "central dogma", explaining new DNA functions to make protein.

1958 Kornberg discovered and isolated DNA polymerase, which became the first enzyme for DNA manipulation in vitro.

1962 Watson and Crick shared the 1962 Nobel Prize for Physiology and Medicine with Maurice Wilkins.

1966 The genetic code was "cracked". Marshall Nierenberg, Heinrich Mathaei, and Ochoa demonstrated that a sequence of three nucleotide bases (codon) determines each of 20 amino acids.

1967 Many Weiss and Howard Green developed somatic cell hybridization where human cells and mouse cells were grown together in one culture.

1970 Howard Terrion and David Baltimore, working independently, first isolated "reverse transcriptase".

1972 Paul Berg isolated and employed a restriction enzyme to cut DNA; he used ligase to join two DNA strands together to form a hybrid circular molecule. This was the first recombinant DNA molecule.

1973 Bruce Ames developed Ames test to identify carcinogenic substances.

1975 Kohler and Milstein fused cells together to produce monoclonal antibodies.

1976 Herbert Boyer and Robert Swanson founded Genetech, Inc., a biotechnology company dedicated to developing and marketing products based on recombinant DNA technology.

THE DAWN OF BIOTECH: 1977 to 2001

1977 Genetech, Inc, reports the production of the first human protein manufactured in a bacteria: somatostatin, a human growth hormone factor.

Maxam and Gilbert devised a method for sequencing DNA using chemicals.

1978 Genetech, Inc, and the City of Hope National Medical Center announced the successful laboratory production of human insulin using recombinant DNA technology.

1979 John Baxter reported cloning the gene for human growth hormone.

1980 Researchers introduced a human gene that codes for the protein interferon into a bacterium.

Kary Mullis invented a technique for multiplying DNA sequences in vitro, i.e. polymerase chain reaction (PCR).

1981 Genetech, Inc, cloned interferon gamma.

First transgenic animals was produced by transferring genes from other animals into mice.

1982 Genetech, Inc, received approval from the FDA to market genetically engineered human insulin.

Applied Biosystems, Inc, introduced the first commercial gas phase protein sequencer, dramatically reducing the amount of protein sample needed for sequencing.

1983 Eli Lilly received a License to make insulin.

1985 Genetically engineered plants resistant to insects, viruses, and bacteria were field tested for the first time.

1986 VC Berkeley and chemist, Peter Schultz described new method to combine antibodies and enzymes creating “abzymes”.

1990 First gene therapy took place, on a 4-year old girl with an Immune system disorder called ADA deficiency.

The Human Genome Project, the international effort to map all of the genes in the human body, was launched.

1994 First genetically engineered food product, the Flavr Savr tomato was produced.

1997 Cloning of Dolly, the sheep by Ian Wilmut.

Artificial human chromosomes created for the first time.

Fellistin, a recombinant follicle stimulating hormone, approved for treatment of infertility.

Complete genomes of *Borrelia burgdorferi, E.Coli and H. Pyroli* were sequenced.

1998 Two research teams succeeded in growing embryonic stem cells.

The first complete genome of C. elegans, a nematode was sequenced.

A rough draft of the human genome map produced, showing the locations of more than 30,000 genes.

Gerhardt and James A. Thomson multiplied human embryonic stem cells.

Neuronal stem cells were discovered.

2000 Human Genome project was reported to be completed.

2001 Human chromosome 20 was sequenced completely.

Animal biotechnology

Cell Fate and Potency

The process underlying the formation of general body plan of an organism is encoded in the genome. The fact that species faithfully transmit their basic characters from parent to offspring is a reflection of this principle of genome coding. During development, cells adopt specific fates, that is, they have the capacity to differentiate into particular kinds of cells. The process of commitment to a particular fate is called DETERMINATION. Cell determination is a gradual process. A cell does not go directly from been totally uncommitted, that is TOTIPOTENT, to been earmarked for a single fate. As zygotic cell division proceeds, periodic decisions are made in each cell lineage to specify more exactly the fate of the daughter cells. Some of the fate decisions are made autonomously by the dividing cell, other decisions are made by committees, in which case the fate if the cell becomes dependent upon the input from neighbouring cells.

By definition, a newly fertilized single-celled zygote is TOTIPOTENT. It could give rise to every and any cell type in the adult.

Biodiversity of farm animals

The 1992 United Nations Earth Summit in Rio de Janeiro, defined biodiversity as:

The variability among living organisms from all sources, including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems

This is in fact the closest we come to a single legally accepted definition of biodiversity, since it is the definition adopted by the United Nations Convention on Biological Diversity. The parties to this convention include all the countries on Earth with the exception of Andorra, Brunei Darussalam, the Holy See, Iraq, Somalia, Timor-Leste, and the United States of America.

If the gene is the fundamental unit of natural selection, and thus of evolution, then the real biodiversity is the genetic diversity as it relates to species diversity.

For geneticists, biodiversity is the diversity of genes and organisms. They study processes such as mutations, gene exchanges, and genome dynamics that occur at the DNA level and generate evolution. For biologists, biodiversity is the diversity of populations of organisms and species, but also the way these organisms function. Organisms appear and disappear; sites are colonized by organisms of the same species or by another. Some species develop social organisations to improve their reproduction goals or use neighbor species that live in communities. Depending on their environment, organisms do not invariably use the same strategies of reproduction.

For ecologists, biodiversity is also the diversity of durable interactions among species. It not only applies to species, but also to their immediate environment (biotope) and the ecoregions that the organisms live in. In each ecosystem, living organisms are part of a whole, they interact with one another, but also with the air, water, and soil that surround them.

Human and Animal Biotechnology

Photo of Dolly by Murdo MacLeod from report by New Scientist
http://www.newscientist.com/nsplus/insight/clone/clone.html.

Cloning

A clone is "a group of cells or an individual organism or group of organisms derived from a single cell. Thus a bacterial colony is a clone, as are indentical twin human beings, as defined by Smith and Wood in their book "Molecular Biology and Biotechnology".

Cloning has come into the spotlight in the media due to the improved techniques and growing concerns about the future applications of this technology and how it may adversely affect our lifestyles.

The Human Genome Project

- From 1990, the U.S. Human Genome Project began as a 15-year effort coordinated by the U.S. Department of Energy and the National Institutes of Health to identify all the estimated 80,000 genes in human DNA and to determine the sequences of the 3 billion chemical bases that make up human DNA, store this information in databases, and develop tools for data analysis. In addition to achieving these goals, researchers also are studying the genetic makeup of several nonhuman organisms. These include the common human gut bacterium *Escherichia coli*, the fruit fly, and the laboratory mouse. A unique aspect of the U.S. Human Genome Project is that it is the first large scientific undertaking to address the ethical, legal, and social issues (ELSI) that may arise from the project."

Genetic Diseases/Disorders

There are over 500 genetic diseases. Many are recessive mutations and they develop only because both parents contribute the same recessive gene to their child. Some common genetic diseases include cystic fibrosis, muscular dystrophy, hemophilia, and sickle-cell anemia.

Animal Biotechnology

Animal biotechnology is the application of scientific and engineering principles to the processing or production of materials by animals or aquatic species to provide goods and services (NRC 2003). Examples of animal biotechnology include the generation of transgenic animals or transgenic fish (animals or fish with one or more genes introduced by human intervention), using gene knockout technology to generate animals in which a specific gene has been inactivated, production of nearly identical animals by somatic cell nuclear transfer (also referred to as clones), or production of infertile aquatic species.

The following methods have been effectively utilized in achieving the objectives of human and animal transformation.

Transgenics

Since the early 1980s, methods have been developed and refined to generate transgenic animals or transgenic aquatic species. For example, transgenic livestock and transgenic

aquatic species have been generated with increased growth rates, enhanced lean muscle mass, enhanced resistance to disease or improved use of dietary phosphorous to lessen the environmental impacts of animal manure. Transgenic poultry, swine, goats, and cattle also have been produced that generate large quantities of human proteins in eggs, milk, blood, or urine, with the goal of using these products as human pharmaceuticals. Examples of human pharmaceutical proteins include enzymes, clotting factors, albumin, and antibodies. The major factor limiting widespread use of transgenic animals in agricultural production systems is the relatively inefficient rate (success rate less than 10 percent) of production of transgenic animals..

Gene Knockout Technology

Animal biotechnology has also been used to knock out or inactivate a specific gene. Knockout technology creates a possible source of replacement organs for humans. The process of transplanting cells, tissues, or organs from one species to another is referred to as "xenotransplantation." Currently, the pig is the major animal being considered as a xenotransplant donor to humans. Unfortunately, pig cells and human cells are not immunologically compatible. Pig cells express a carbohydrate epitope (alpha1, 3 galactose) on their surface that is not normally found on human cells. Humans will generate antibodies to this epitope, which will result in acute rejection of the xenograft. Genetic engineering is used to knock out or inactivate the pig gene (alpha1, 3 galactosyl transferase) that attaches this carbohydrate epitope on pig cells. Other examples of knockout technology in animals include inactivation of the prion-related peptide (PRP) gene that may generate animals resistant to diseases associated with prions (bovine spongiform encephalopathy [BSE], Creutzfeldt-Jakob Disease [CJD], scrapie, etc.). Most of these projects are conducted by private companies or in academic laboratories supported by the National Institutes of Health.

Somatic Cell Nuclear Transfer

Another application of animal biotechnology is the use of somatic cell nuclear transfer to produce multiple copies of animals that are nearly identical copies of other animals (transgenic animals, genetically superior animals, or animals that produce high quantities of milk or have some other desirable trait, etc.). This process has been referred to as cloning. To date, somatic cell nuclear transfer has been used to clone cattle, sheep, pigs, goats, horses, mules, cats, rats, and mice. The technique involves culturing somatic cells from an appropriate tissue (fibroblasts) from the animal to be cloned. Nuclei from the cultured somatic cells are then microinjected into an

enucleated oocyte obtained from another individual of the same or a closely related species. Through a process that is not yet understood, the nucleus from the somatic cell is reprogrammed to a pattern of gene expression suitable for directing normal development of the embryo. After further culture and development in vitro, the embryos are transferred to a recipient female and ultimately will result in the birth of live offspring.

The success rate for propagating animals by nuclear transfer is often less than 10 percent and depends on many factors, including the species, source of the recipient ova, cell type of the donor nuclei, treatment of donor cells prior to nuclear transfer, the techniques employed for nuclear transfer, etc..

Production of Infertile Aquatic Species.

In aquaculture production systems, some species are not indigenous to a given area and can pose an ecological risk to native species should the foreign species escape confinement and enter the natural ecosystem. Generation of large populations of sterile fish or mollusks is one potential solution to this problem. Techniques have been developed to alter the chromosome complement to render individual fish and mollusks infertile. For example, triploid individuals (with three, instead of two, sets of chromosomes) have been generated by using various procedures to interfere with the final step in meiosis (extrusion of the second polar body). Timed application of high or low temperatures, various chemicals, or high hydrostatic pressure to newly fertilized eggs has been effective in producing triploid individuals. At a later time, the first cell division of the zygote can be suppressed to produce a fertile tetraploid individual (four sets of chromosomes). Tetraploids can then be mated with normal diploids to produce large numbers of infertile triploids. Unfortunately, in a commercial production system, it is often difficult to obtain sterilization of 100 percent of the individuals; thus, alternative methods are needed to ensure reproductive confinement of transgenic fish. Another technique that is being developed for finfish is to farm monosex fish stocks. Monosex populations can be produced by gender reversal and progeny testing to identify XX males for producing all female stocks or YY males for producing all male stocks.

Problems and Potentials

As with any new technology, animal biotechnology faces a variety of uncertainties, safety issues and potential risks. For example, concerns have been raised regarding:

- the use of unnecessary genes in constructs used to generate transgenic animals,
- the use of vectors with the potential to be transferred or to otherwise contribute sequences to other organisms,
- the potential effects of genetically modified animals on the environment,
- the effects of the biotechnology on the welfare of the animal, and
- potential human health and food safety concerns for meat or animal products derived from animal biotechnology.

Before animal biotechnology will be used widely by animal agriculture production systems, additional research will be needed to determine if the benefits of animal biotechnology outweigh these potential risks. The USDA Biotechnology Risk Assessment Grants program supports environmental risk assessment research projects on genetically engineered animals. In addition, the NRI Animal Protection program supports research projects to determine the effects of genetic modification on the health and well-being of the animal.

Advances in animal biotechnology have been facilitated by recent progress in sequencing and analyzing animal genomes, identification of molecular markers (microsatellites, expressed sequence tags [ESTs], quantitative trait loci [QTLs], etc.) and a better understanding of the mechanisms that regulate gene expression.

For more information on these topics and projects supported by CSREES in this area, see Animal Breeding, Genetics, and Genomics.

CHROMOSOMES AND GENES

STRUCTURAL GENES

Structural gene control

GENE BANKS AND LIBRARIES

GENE CLONING

ISOLATING GENES

GENE SPLICING

What is DNA?

We all know that elephants only give birth to little elephants, giraffes to giraffes, dogs to dogs and so on for every type of living creature. But why is this so?

The answer lies in a molecule called deoxyribonucleic acid (DNA), which contains the biological instructions that make each species unique. DNA, along with the instructions it contains, is passed from adult organisms to their offspring during reproduction ever so faithfully.

Where is DNA found?

DNA is found inside a special area of the cell called the **nucleus**. Because the cell is very small, and because organisms have many DNA molecules per cell, each DNA molecule must be tightly packaged. This packaged form of the DNA is called a chromosome.

DNA spends a lot of time in its chromosome form. But during cell division, DNA unwinds so it can be copied and the copies transferred to new cells. DNA also unwinds so that its instructions can be used to make proteins and for other biological processes.

Researchers refer to DNA found in the cell's nucleus as **nuclear DNA**. An organism's complete set of nuclear DNA is called its **genome**.

Besides the DNA located in the nucleus, humans and other complex organisms also have a small amount of DNA in cell structures known as **mitochondria**. Mitochondria generate the energy the cell needs to function properly.

In sexual reproduction, organisms inherit half of their nuclear DNA from the male parent and half from the female parent. However, organisms inherit all of their mitochondrial DNA from the female parent. This occurs because only egg cells, and not sperm cells, keep their mitochondria during fertilization. The mitochondria of the male parent is carried on the tail of the spermatozoa and it is lost during fertilization, hence it can not be transferred to the offspring.

What is DNA made of?

DNA is made of chemical building blocks called **nucleotides**. These building blocks are made of three parts: a phosphate group, a sugar group and one of four types of nitrogen bases. To form a strand of DNA, nucleotides are linked into chains, with the phosphate and sugar groups alternating.

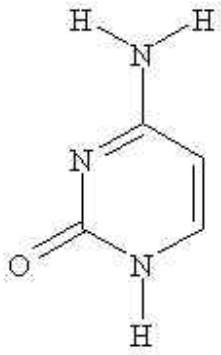
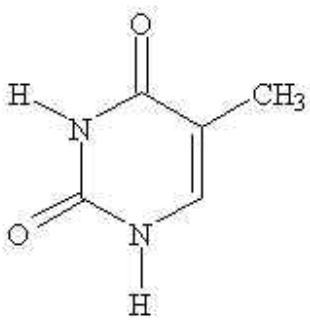
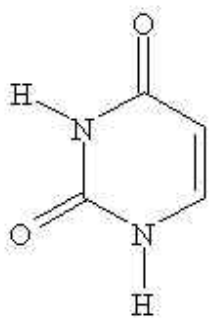
The four types of **nitrogen bases** called heterocyclic bases found in nucleotides are: adenine (A), thymine (T), guanine (G) and cytosine (C).

Heterocyclic Bases

Several different bases are found in nucleotides. They are heterocyclic bases or sometimes referred to as nitrogenous bases because they contain nitrogen within the rings. The fact that they are bases is actually irrelevant for the function that they serve and we really won't be paying attention to their base properties. (Structures for these compounds are also shown at the top of Example 4 in your workbook.)

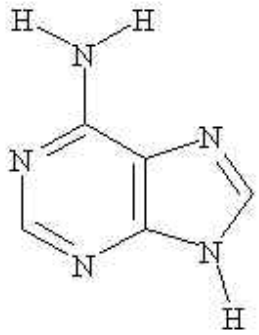
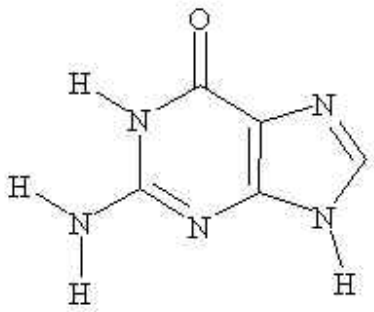
Pyrimidines

Some have **one ring** and are similar in structure to the compound **pyrimidine** and, because of that, they are called the pyrimidines or the pyrimidine bases. There are three of them and they are called **cytosine** which is found in both DNA and RNA, **thymine**, which is found only in DNA, and **uracil**, which is found only in RNA. The abbreviations **C**, **T** and **U** will be used extensively to refer to these compounds.

		
cytosine (C)	**thymine (T)**	**uracil (U)**

Purines

Some of these heterocyclic bases have **two rings** like the compound **purine** and, therefore, they are called the purines or the purine bases. They are **adenine** and **guanine**, represented by the letters **A** and **G**, and they are both found in DNA and RNA.

	
adenine (A)	**guanine (G)**

The order, or sequence, of these bases determines what biological instructions are contained in a strand of DNA. For example, the

sequence ATCGTT might instruct for blue eyes, while ATCGCT might instruct for brown.

Each DNA sequence that contains instructions to make a protein is known as a **gene**. The size of a gene may vary greatly, ranging from about **1,000 bases to 1 million bases** in humans.

The complete **DNA instruction book, or genome**, for a human contains about **3 billion bases** and about **20,000 genes** on **23 pairs of chromosomes**.

What does DNA do?

DNA contains the instructions needed for an organism to develop, survive and reproduce. To carry out these functions, DNA sequences must be converted into messages that can be used to produce proteins, which are the complex molecules that do most of the work in our bodies.

How are DNA sequences used to make proteins?

DNA's instructions are used to make proteins in a two-step process. First, **enzymes read** the information in a DNA molecule and **transcribe** it into an intermediary molecule called **messenger ribonucleic acid, or mRNA**.

Next, the information contained in the mRNA molecule is **translated** into the **"language" of amino acids**, which are the building blocks of proteins. This language tells the cell's protein-making machinery the precise order in which to link the amino acids to produce a specific protein. This is a major task because there are **20 types of amino**

acids, which can be placed in many different orders to form a wide variety of proteins.

Who discovered DNA?

The German biochemist Frederich Miescher first observed DNA in the late 1800s. But nearly a century passed from that discovery until researchers unraveled the structure of the DNA molecule and realized its central importance to biology.

For many years, scientists debated which molecule carried life's biological instructions. Most thought that DNA was too simple a molecule to play such a critical role. Instead, they argued that proteins were more likely to carry out this vital function because of their greater complexity and wider variety of forms.

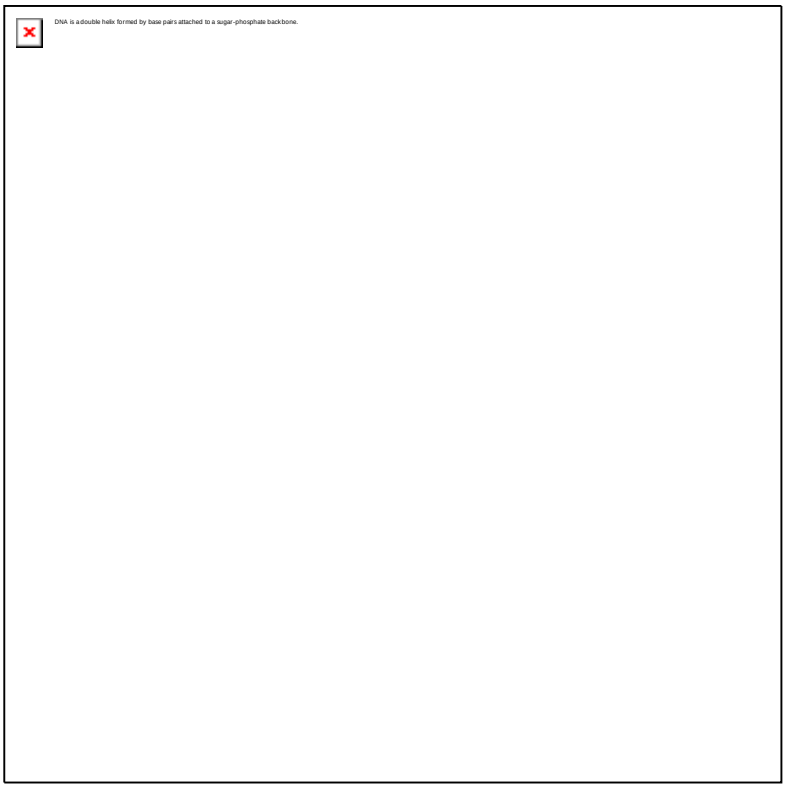
The importance of DNA became clear in 1953 through the work of James Watson, Francis Crick, Maurice Wilkins and Rosalind Franklin, who used X-ray diffraction patterns and building models to figure out the double helix structure of DNA - a structure that enables it to carry biological information from one generation to the next.

What is the DNA double helix?

Scientist use the term "double helix" to describe DNA's winding, two-stranded chemical structure. This shape - which looks much like a twisted ladder - gives DNA the power to pass along biological instructions with great precision.

To understand DNA's double helix from a chemical standpoint, we need to picture the sides of the ladder as strands of alternating sugar

and phosphate groups - strands that run in opposite directions. Each "rung" of the ladder is made up of two nitrogen bases, paired together by hydrogen bonds. Because of the highly specific nature of this type of chemical pairing, base A always pairs with base T, and likewise C with G. So, if you know the sequence of the bases on one strand of a DNA double helix, it is a simple matter to figure out the sequence of bases on the other strand.


DNA is a double helix formed by base pairs attached to a sugar-phosphate backbone.

DNA is a double helix formed by base pairs attached to a sugar-phosphate backbone.

DNA's unique structure enables the molecule to copy itself during cell division. When a cell prepares to divide, the DNA helix splits down the middle and becomes two single strands. These single strands serve as templates for building two new, double-stranded DNA molecules - each a replica of the original DNA molecule. In this process, an A base is added wherever there is a T, a C where there is a G, and so on until all of the bases once again have partners.

In addition, when proteins are being made, the double helix unwinds to allow a single strand of DNA to serve as a template. This template strand is then transcribed into mRNA, which is a molecule that conveys vital instructions to the cell's protein-making machinery.

The Genetic Code

Genome sizes

DNA Reassociation and Complexity

Complementary DNA

Restriction Enzymes

Proteins and Amino acids

GENOME MAPPING:

For several years scientists have been faced with the problem of searching for a specific gene somewhere within the vast human genome. In this search they have available to them two broad categories of maps: **genetic maps** and **physical maps**. Both genetic and physical maps provide the likely order of items along a chromosome. However, a genetic map, like an interstate highway map, provides an indirect estimate of the distance between two items and is limited to ordering certain items. One could say that genetic maps serve to guide a scientist toward a gene, just like an interstate map guides a driver from city to city. On the other hand, physical maps mark an estimate of the true distance, in measurements called **base pairs**, between items of interest. To continue our analogy, physical maps would then be similar to street maps, where the distance between two sites of interest may be defined more precisely in terms of city blocks or street addresses. Physical maps, therefore, allow a scientist to more easily home in on the location of a gene. An appreciation of how each of these maps is constructed may be helpful in understanding how scientists use these maps to traverse that genetic highway commonly referred to as the "human genome".

GENETIC MAPS

Genetic maps serve to guide a scientist toward a gene, just like an interstate map guides a driver from city to city. Physical maps are more similar to street maps and allow a scientist to more easily home-in on a gene's location.

Types of Landmarks Found on a Genetic Map

Just like interstate maps have cities and towns that serve as landmarks, genetic maps have landmarks known as **genetic markers**, or "markers" for short. The term marker is used very broadly to describe any observable variation that results from an alteration, or mutation, at a single genetic locus. A marker may be used as one landmark on a map if, in most cases, that stretch of DNA is inherited from parent to child according to the standard rules of inheritance. Markers can be within genes that code for a noticeable physical characteristic such as eye color, or a not so noticeable trait such as a disease. **DNA-based reagents** can also serve as markers. These types of markers are found within the non-coding regions of genes and are used to detect unique regions on a chromosome. DNA markers are especially useful for generating genetic maps when there are occasional, predictable mutations that occur during **meiosis**—the formation of gametes such as egg and sperm—that, over many generations, lead to a high degree of variability in the DNA content of the marker from individual to individual.

Genetic maps use landmarks called genetic markers to guide researchers on their gene hunt.

COMMONLY USED DNA MARKERS

- **RFLPs**, or **restriction fragment length polymorphisms**, were among the first developed DNA markers. RFLPs are defined by the presence or absence of a specific site, called a restriction site, for a bacterial restriction enzyme. This enzyme breaks apart strands of DNA wherever they contain a certain nucleotide sequence.

- **VNTRs**, or **variable number of tandem repeat polymorphisms**, occur in non-coding regions of DNA. This type of marker is defined by the presence of a nucleotide sequence that is repeated several times. In each case, the number of times a sequence is repeated may vary.

- **Microsatellite polymorphisms** are defined by a variable number of repetitions of a very small number of base pairs within a sequence. Oftentimes, these repeats consist of the nucleotides, or bases, cytosine and adenosine. The number of repeats for a given microsatellite may differ between individuals, hence the term **polymorphism**--the existence of different forms within a population.

- **SNPs**, or **single nucleotide polymorphisms**, are individual point mutations, or substitutions of a single nucleotide, that do not change the overall length of the DNA sequence in that region. SNPs occur throughout an individual's genome.

Linkage Analysis and Genetic Mapping

Early geneticists recognized that genes are located on chromosomes and believed that each individual chromosome was inherited as an intact unit. They hypothesized that if two genes were located on the same chromosome, they were physically linked together and were inherited together. We now know that this is not always the case. Studies conducted around 1910 demonstrated that very few pairs of genes displayed complete linkage. Pairs of genes were either inherited independently or displayed **partial linkage**—that is, they were inherited together sometimes, but not always.

During **meiosis**—the process whereby gametes (eggs and sperm) are produced— two copies of each chromosome pair become physically close. The chromosome arms can then undergo breakage and exchange segments of DNA, a process referred to as recombination or crossing-over. If recombination occurs, each chromosome found in the gamete will consist of a "mixture" of material from both members of the chromosome pair. Thus, recombination events directly affect the inheritance pattern of those genes involved.

It is the behavior of chromosomes during meiosis that determines whether two genes will remain linked.

Because one cannot physically see crossover events, it is difficult to determine with any degree of certainty how many crossovers have actually occurred. But, using the phenomenon of **co-segregation of alleles of nearby markers**, researchers can reverse-engineer meiosis and identify markers that lie close to each other. Then, using a statistical technique called **genetic linkage analysis**, researchers can infer a likely crossover pattern, and from that an order of the markers involved. Researchers can also infer an estimate for the probability that a recombination occurs between each pair of markers.

An allele is one of the variant forms of a DNA sequence at a particular locus, or location, on a chromosome. Co-segregation

of alleles refers to the movement of each marker during meiosis. If a marker tends to "travel" with the disease status, the markers are said to co-segregate.

If recombination occurs as a random event, then two markers that are close together should be separated less frequently than two markers that are more distant from one another. The recombination probability between two markers, which can range from 0 to 0.5, increases monotonically as the distance between the two markers increases along a chromosome. Therefore, the recombination probability may be used as a surrogate for ordering genetic markers along a chromosome. If you then determine the recombination frequencies for different pairs of markers, you can construct a map of their relative positions on the chromosome. But alas, predicting recombination is not so simple. Although crossovers are random, they are not uniformly distributed across the genome or any chromosome. Some chromosomal regions, called recombination hotspots, are more likely to be involved in crossovers than other regions of a chromosome. This means that genetic map distance does not always indicate physical distance between markers. Despite these qualifications, linkage analysis usually correctly deduces marker order, and distance estimates are sufficient to generate genetic maps that can serve as a valuable framework for genome sequencing.
Monotonic functions are functions that tend to move only in one direction.

Linkage maps can tell you where markers are in relation to each other on the chromosome, but the actual "mileage" between those markers may not be so well defined.

Linkage Studies in Patient Populations: Genetic Maps and Gene Hunting

In humans, genetic diseases are frequently used as gene markers, with the disease state being one allele and the healthy state the second allele.
In humans, data for calculating recombination frequencies are obtained by examining the genetic makeup of the members of successive generations of existing families, termed **human pedigree analysis**. Linkage studies begin

by obtaining blood samples from a group of related individuals. For relatively rare diseases, scientists find a few large families that have many cases of the disease and obtain samples from as many family members as possible. For more common diseases where the pattern of disease inheritance is unclear, scientists will identify a large number of affected families and will take samples from four to thirty close relatives. DNA is then harvested from all of the blood samples and screened for the presence, or **co-inheritance**, of two markers. One marker is usually the gene of interest, generally associated with a physically identifiable characteristic. The other is usually one of the various detectable rearrangements mentioned earlier, such as a microsatellite. A computerized analysis is then performed to determine whether the two markers are linked and approximately how far apart those markers are from one another. In this case, the value of the genetic map is that an inherited disease can be located on the map by following the inheritance of a DNA marker present in affected individuals but absent in unaffected individuals, although the molecular basis of the disease may not yet be understood, nor the gene(s) responsible identified.

Genetic Maps as a Framework for Physical Map Construction

Genetic maps are also used to generate the essential backbone, or scaffold, needed for the creation of more detailed human genome maps. These detailed maps, called **physical maps**, further define the DNA sequence between genetic markers and are essential to the rapid identification of genes.

PHYSICAL MAPS

Types of Physical Maps and What They Measure

Physical maps can be divided into three general types: **chromosomal** or **cytogenetic maps**, **radiation hybrid (RH) maps**, and **sequence maps**. The different types of maps vary in their degree of **resolution**, that is, the ability to measure the separation of elements that are close together. The higher the resolution, the better the picture.

The lowest-resolution physical map is the chromosomal or **cytogenetic map**, which is based on the distinctive banding patterns observed by light microscopy of stained chromosomes. As with genetic linkage mapping, chromosomal mapping can be used to locate genetic markers defined by traits observable only in whole organisms. Because chromosomal maps are

based on estimates of physical distance, they are considered to be physical maps. Yet, the number of base pairs within a band can only be estimated.

RH maps and sequence maps, on the other hand, are more detailed. RH maps are similar to linkage maps in that they show estimates of distance between genetic and physical markers, but that is where the similarity ends. **RH maps** are able to provide more precise information regarding the distance between markers than can a linkage map.

The physical map that provides the most detail is the sequence map. **Sequence maps** show genetic markers, as well as the sequence between the markers, measured in base pairs.

How Are Physical Maps Made and Used?

RH Mapping

RH mapping, like linkage mapping, shows an estimated distance between genetic markers. But, rather than relying on natural recombination to separate two markers, scientists use breaks induced by radiation to determine the distance between two markers. In RH mapping, a scientist exposes DNA to measured doses of radiation, and in doing so, controls the average distance between breaks in a chromosome. By varying the degree of radiation exposure to the DNA, a scientist can induce breaks between two markers that are very close together. The ability to separate closely linked markers allows scientists to produce more detailed maps. RH mapping provides a way to localize almost any genetic marker, as well as other genomic fragments, to a defined map position, and RH maps are extremely useful for ordering markers in regions where highly **polymorphic genetic markers** are scarce. Scientists also use RH maps as a bridge between linkage maps and sequence maps. In doing so, they have been able to more easily identify the location(s) of genes involved in diseases such as spinal muscular atrophy and hyperekplexia, more commonly known as "startle disease".

Polymorphic refers to the existence of two or more forms of the same gene, or genetic marker, with each form being too common in a population to be merely attributable to a new mutation. Polymorphism

is a useful genetic marker because it enables researchers to sometimes distinguish which allele was inherited.

Sequence Mapping

Sequence tagged site (STS) mapping is another physical mapping technique. An STS is a short DNA sequence that has been shown to be unique. To qualify as an STS, the exact location and order of the bases of the sequence must be known, and this sequence may occur only once in the chromosome being studied or in the genome as a whole if the DNA fragment set covers the entire genome.

COMMON SOURCES OF STSs

- **Expressed sequence tags (ESTs)** are short sequences obtained by analysis of complementary DNA (cDNA) clones. Complementary DNA is prepared by converting mRNA into double-stranded DNA and is thought to represent the sequences of the genes being expressed. An EST can be used as an STS if it comes from a unique gene and not from a member of a gene family in which all of the genes have the same, or similar, sequences.
- **Simple sequence length polymorphisms (SSLPs)** are arrays of repeat sequences that display length variations. SSLPs that are polymorphic and have already been mapped by linkage analysis are particularly valuable because they provide a connection between genetic and physical maps.

- **Random genomic sequences** are obtained by sequencing random pieces of cloned genomic DNA or by examining sequences already deposited in a database.

To map a set of STSs, a collection of overlapping DNA fragments from a chromosome is digested into smaller fragments using **restriction enzymes**, agents that cut up DNA molecules at defined target points. The data from which the map will be derived are then obtained by noting which fragments contain which STSs. To accomplish this, scientists copy the DNA fragments using a process known as "**molecular cloning**". Cloning involves the use of a special technology, called **recombinant DNA technology**, to copy DNA fragments inside a foreign host.

First, the fragments are united with a carrier, also called a vector. After introduction into a suitable host, the DNA fragments can then be reproduced along with the host cell DNA, providing unlimited material for experimental study. An unordered set of cloned DNA fragments is called a **library**.

Next, the **clones**, or copies, are assembled in the order they would be found in the original chromosome by determining which clones contain overlapping DNA fragments. This assembly of overlapping clones is called a **clone contig**. Once the order of the clones in a chromosome is known, the clones are placed in frozen storage, and the information about the order of the clones is stored in a computer, providing a valuable resource that may be used for further studies. These data are then used as the base material for generating a lengthy, continuous DNA sequence, and the STSs serve to anchor the sequence onto a physical map.

The Need to Integrate Physical and Genetic Maps

As with most complex techniques, STS-based mapping has its limitations. In addition to gaps in clone coverage, DNA fragments may become lost or mistakenly mapped to a wrong position. These errors may occur for a variety of reasons. A DNA fragment may break, resulting in an STS that maps to a different position. DNA fragments may also get deleted from a clone during the replication process, resulting in the absence of an STS that should be present. Sometimes a clone composed of DNA fragments from two distinct genomic regions is replicated, leading to DNA segments that are widely separated in the genome being mistakenly mapped to adjacent positions. Lastly, a DNA fragment may become contaminated with host genetic material, once again leading to an STS that will map to the wrong location. To help overcome these problems, as well as to improve overall mapping accuracy, researchers have begun comparing and integrating STS-based physical maps with genetic, RH, and cytogenetic maps. Cross-referencing different genomic maps enhances the utility of a given map, confirms STS order, and helps order and orient evolving contigs.

NCBI and Map Integration

Comparing the many available genetic and physical maps can be a time-consuming step, especially when trying to pinpoint the location of a new gene. Without the use of computers and special software designed to align the various maps, matching a sequence to a region of a chromosome that

corresponds to the gene location would be very difficult. It would be like trying to compare 20 different interstate and street maps to get from a house in Ukiah, California to a house in Beaver Dam, Wisconsin. (a house in Sokoto to another in Calabar). You could compare the maps yourself and create your own travel itinerary, but it would probably take a long time. Wouldn't it be easier and faster to have the automobile club create an integrated map for you? That is the goal behind NCBI's Human Genome Map Viewer.

NCBI's Map Viewer: A Tool for Integrating Genetic and Physical Maps

The NCBI Map Viewer provides a graphical display of the available human genome sequence data as well as sequence, cytogenetic, genetic linkage, and RH maps. Map Viewer can simultaneously display up to seven maps, selected from a large set of maps, and allows the user access to detailed information for a selected map region. Map Viewer uses a common sequence numbering system to align sequence maps and shared markers as well as gene names to align other maps. You can use NCBI's Map Viewer to search for a gene in a number of genomes, by choosing an organism from the Map Viewer home page.

Map Viewer Getting Started

Need help using the NCBI Map Viewer? Try GETTING STARTED, a quick "how-to guide" on NCBI data mining tools designed for the novice user. GETTING STARTED using Map Viewer provides:

- information on how you can use Map Viewer
- descriptions of the Map Viewer layout
- step-by-step information on using the Map Viewer

i.e. shortcuts for getting to where you need to go

Can genes be turned on and off in cells?

Each cell expresses, or turns on, only a fraction of its genes. The rest of the genes are repressed, or turned off. The process of turning genes on and off is known as gene regulation. Gene regulation is an important part of normal development. Genes are turned on and off in different patterns during development to make a brain cell look and act different from a liver cell or a muscle cell, for example. Gene regulation also allows cells to react quickly

to changes in their environments. Although we know that the regulation of genes is critical for life, this complex process is not yet fully understood.

Gene regulation can occur at any point during gene expression, but most commonly occurs at the level of transcription (when the information in a gene's DNA is transferred to mRNA). Signals from the environment or from other cells activate proteins called transcription factors. These proteins bind to regulatory regions of a gene and increase or decrease the level of transcription. By controlling the level of transcription, this process can determine the amount of protein product that is made by a gene at any given time.

MICROARRAYS: CHIPPING AWAY AT THE MYSTERIES OF SCIENCE AND MEDICINE

The proper and harmonious expression of a large number of genes is a critical component of normal growth and development and the maintenance of proper health. Disruptions or changes in gene expression are responsible for many diseases.

With only a few exceptions, every cell of the body contains a full set of chromosomes and identical genes. Only a fraction of these genes are turned on, however, and it is the subset that is "**expressed**" that confers unique properties to each cell type. "**Gene expression**" is the term used to describe the transcription of the information contained within the **DNA**, the repository of genetic information, into messenger RNA (mRNA) molecules that are then translated into the proteins that perform most of the critical functions of cells. Scientists study the kinds and amounts of mRNA produced by a cell to learn which genes are expressed, which in turn provides insights into how the cell responds to its changing needs. Gene expression is a highly complex and tightly regulated process that allows a cell to respond dynamically both to environmental stimuli and to its own changing needs. This mechanism acts as both an "**on/off**" **switch** to control which genes are expressed in a cell as well as a "**volume control**" that increases or decreases the level of expression of particular genes as necessary.

ENABLING TECHNOLOGIES

Biomedical research evolves and advances not only through the compilation of knowledge but also through the development of new

technologies. Using traditional methods to assay gene expression, researchers were able to survey a relatively small number of genes at a time. The emergence of new tools enables researchers to address previously intractable problems and to uncover novel potential targets for therapies. Microarrays allow scientists to analyze expression of many genes in a single experiment quickly and efficiently. They represent a major methodological advance and illustrate how the advent of new technologies provides powerful tools for researchers. Scientists are using microarray technology to try to understand fundamental aspects of growth and development as well as to explore the underlying genetic causes of many human diseases.

DNA Microarrays: The Technical Foundations

Two recent complementary advances, one in knowledge and one in technology, are greatly facilitating the study of gene expression and the discovery of the roles played by specific genes in the development of disease. As a result of the Human Genome Project, there has been an explosion in the amount of information available about the DNA sequence of the human genome. Consequently, researchers have identified a large number of novel genes within these previously unknown sequences. The challenge currently facing scientists is to find a way to organize and catalog this vast amount of information into a usable form. Only after the functions of the new genes are discovered will the full impact of the Human Genome Project be realized.

The second advance may facilitate the identification and classification of this DNA sequence information and the assignment of functions to these new genes: the emergence of DNA microarray technology. A microarray works by exploiting the ability of a given mRNA molecule to bind specifically to, or hybridize to, the DNA template from which it originated. By using an array containing many DNA samples, scientists can determine, in a single experiment, the expression levels of hundreds or thousands of genes within a cell by measuring the amount of mRNA bound to each site on the array. With the aid of a computer, the amount of mRNA bound to the spots on the microarray is precisely measured, generating a profile of gene expression in the cell.

A **microarray** is a tool for analyzing gene expression that consists of a small membrane or glass slide containing samples of many genes arranged in a regular pattern.

Why Are Microarrays Important?

Microarrays are a significant advance both because they may contain a very large number of genes and because of their small size. Microarrays are therefore useful when one wants to survey a large number of genes quickly or when the sample to be studied is small. Microarrays may be used to assay gene expression within a single sample or to compare gene expression in two different cell types or tissue samples, such as in healthy and diseased tissue. Because a microarray can be used to examine the expression of hundreds or thousands of genes at once, it promises to revolutionize the way scientists examine gene expression. This technology is still considered to be in its infancy; therefore, many initial studies using microarrays have represented simple surveys of gene expression profiles in a variety of cell types. Nevertheless, these studies represent an important and necessary first step in our understanding and cataloging of the human genome.

As more information accumulates, scientists will be able to use microarrays to ask increasingly complex questions and perform more intricate experiments. With new advances, researchers will be able to infer probable functions of new genes based on similarities in expression patterns with those of known genes. Ultimately, these studies promise to expand the size of existing gene families, reveal new patterns of coordinated gene expression across gene families, and uncover entirely new categories of genes. Furthermore, because the product of any one gene usually interacts with those of many others, our understanding of how these genes coordinate will become clearer through such analyses, and precise knowledge of these inter-relationships will emerge. The use of microarrays may also speed the identification of genes involved in the development of various diseases by enabling scientists to examine a much larger number of genes. This technology will also aid the examination of the integration of gene expression and function at the cellular level, revealing how multiple gene products work together to produce physical and chemical responses to both static and changing cellular needs.

(from http://www.ornl.gov/TechResources/Human_Genome/home.html)

- Current news: http://www.ornl.gov/TechResources/Human_Genome/home.html
- Issues raised: http://www.ornl.gov/hgmis/publicat/genechoice/index.html

Human Genome Program-- Department of Energy:
http://www.er.doe.gov/production/ober/hug_top.html

- Genetic diseases in children:
  http://mcrcr4.med.nyu.edu/~murphp01/homenew.html
- Murdoch Institute: http://murdoch.rch.unimelb.edu.au
- Center for Inherited Disorders of Energy Metabolism:
  http://www.cwru.edu/med/CIDEM/cidem.htm

Online Mendelian Inheritance in Man--OMIM: http://www3.ncbi.nlm.nih.gov/omim/

GENE SYNTHESIS

GENE SWITCHING

RECOMBINANT DNA AND SOCIAL RESPONSIBILITIES

BLOOD GROUPS

MULTIPLE ALLELES

DNA Extraction

Introduction to RNA extraction

Brief Description of procedure.

- Switch on the Lamina Flow
- Wipe the work top with 70% Ethanol followed by distilled H2O and RNase Away
- Arrange on work top all equip and reagents for the day's work i.e.

1. 200, 1000ul pipette tips
2. 2-20; 20-200; 100 – 1000 micropipettes
3. RNase Away; 75% Ethanol, 100% ethanol; Chloroform; Isopropanol; Trizol® Reagent; HPLC grade water; Sterile Scalpel Blades; Omni International Tissue Master; sterile cryo tubes; corning tubes; autoclaved microtubes, get the 50ul of Formamide in DEPC water 50:50
4. Gloves; KIM wipes
5. Thermoline flask with Liquid Nitrogen
6. refrigerated micro centrifuge in cold room (-4^{o}C)
7. IJJB089y work book- (yellow manual, page 89 labelled IJJB)

- Wear your gloves (single or double)
- Label 2 cryo tubes; into one add 1ml of HPLC grade water; into the second add 1ml of Trizol
- Label 2 microtubes leave empty
- Label 2 15ml corning tubes, into one add 5ml HPLC grade water; into the second add 3mls Trizol
- Get 2 petri dishes, into one place Kim wipe pour on it 2ml RNase Away; Open a sterile scalpel blade carefully drop it onto the wet Kim wipe make sure the holding edge is on the edge of the Petri dish (may open the second on it as well and cover immediately with the edge of the wet Kim wipe and the Petri dish lid. Into the second Petri dish pour 1000ul of Trizol and cover.
- Connect the Tissue Master to the Hood mains, sterilize it by flicking the tip inside the 5ml HPLC water several times and then inside the 3ml Trizol solution inside the large Corning tubes.
- Pour 100ul of Trizol into a cryotube
- Pour enough quantity of liquid N2 from Thermo flask into Polystyrene box ready for use.
- Take one corning tube of Broiler sample from Liquid N2 Tank labeled 049W. This is a 20d.p.h. broiler muscle. (label = Broiler 20d.p.h)
- Drop bottle in liquid Nitrogen and as your sampling progresses always ensure that the corning tube is dropped into Liquid Nitrogen not necessarily covered when sample is being taken from the tube
- Take about 50ng of muscle from the tube with a scalpel into the Trizol in the Petri dish. Finely chop it and mix with the Trizol.
- Suck all the mixture into the 100ul Trizol in cryo tube.
- Homogenize sample with the tissue master very well.
- Keep at room temp for 5-10 mins

Should extra muscle drop into the Trizol don't return it into the sample tube, rather put it into a cryo tube and drop into liquid Nitrogen in the polystyrene because it contains Trizol

- Return the remaining muscle sample into the LN2 tank
- **Wipe your hand with the RNase Away. Do this as often as you touch anything before touching your sample**

because RNA is all around you, from your forehead and bacteria around. Take Absolute precaution

- **Label a corning tube from the -20^{o}C fridge IJJB089y, pour into it 50ul of 50:50 DEPC Water with formamide as the tube into which you will reconstitute your RNA after extraction.**
- **If the mixture is more than 1.5 mls, reduce volume by transferring only 1ml into Cryo tube allow to rest for 5 mins.**
- **Spin down at 15,000rpm for 10mins in a refridgerated Microcentrifuge inside the cold room at -4^{o}C**
- **While waiting, clean and rinse your homogenizer in the HPLC Water, wipe, cover and disconnect and put away.**
- **Check your sample, remove the supernatant (pink layer only) into another cryo tube. Reduce the volume if it is too full to be able to accommodate 200ul of chloroform without spilling over.**
- **Add 200ul of chloroform, mix well by hand**
- **Leave at room temp for 5-10 mins, the supernatant begins to separate**
- **Spin down at 15,000 rpm for 15mins at -4^{o}C in the cold room**
- **Remove clear supernatant into a new tube, if you mistakenly suck up the trizol with the liquid, re spin down again for 2 mins**
- **Get as much of the supernatant if it means using 10ul pipette.**
- **Add 0.5ml (500ul) isopropanol**
- **Leave at room temp for 5-10mins**
- **Spin down at 15,000 rpm for 18 mins**
- **Discard isopropanol layer supernatant**
- **Wash pellet with 1ml 75% ethanol**
- **Briefly vortex**
- **Spin down at 15,000 for 5 mins at -4^{o}C**
- **Discard supernatant by decanting (decanting means pouring gently across a Kimwipe) followed by one flick**
- **Wash pellet again with 1 ml of 75% ethanol**
- **Briefly vortex**
- **Spin down at 15,000 rpm for 5 mins at -4^{o}C**
- **Decant supernatant and place tube upside down on Kim Wipe rest tube by the side of the pack**
- **Leave for 10-15 mins, never exceed 15 mins because if it overdries it will never come off the tube.**

- **Re suspend in 50ul of 50:50 DEPC water and Formamide already in the labeled corning tube.**
- **Should you make a mistake, re constitute the solution with 1ml 100% ethanol**
- **Spin down as with DNA, decant supernatant and re suspend airdried RNA in 50ul 50:50 DEPC/Formamide.**
- **Label properly and store away in sample box inside -20^{o}C fridge**

8th May 2009

Run Agarose gel of sample on 1JJB089y (049W broiler 20d.p.h)

- **Bring out the Agarose gel elecrophoresis system**
- **Pour into the tank enough 1 X TAE buffer to cover the running plate**
- **Bring out the already prepared gel from the cold room (note prepared 7% agarose gel could be kept at -4^{o}C in plastic container ready for use)**
- **Cut the number of lanes required in the middle but with half cut comb edges note that an extra lane must be available for the RNA ladder.**
- **Place on the gel plate covered by the buffer.**
- **Rinse the wells with the buffer**
- **Drop 3ul of loading dye for the number of samples and the ladder on a cut strip of parafilm**
- **Bring out the RNA ladder lot # 414498 from the -20^{o}C fridge**
- **Pipette out 3ul of ladder mix thoroughly with the drop of loading buffer, suck up, rinse the pipette tip with the buffer in the tank, load into middle or noted well on your diagram in your notepad**
- **Take also 3ul of the sample, mix thoroughly with the 3ul loading buffer, suck up, clean the pipette tip in the buffer and load into the well**
- **Cover and Connect the tank (black to black, red to red) and to the power pack.**
- **Switch on the pack, set to 140volts ;400mAmp.**
- **Check your time record the start: 12.15**
- **Allow gel to run till almost end of the gel following the track of the runs**
- **Stop time =12.44 approx. 30mins run.**
- **Stop and view under UV light on the UV box**
- **Take sample in polytene bag to Animal Science (Dr Hughes lab) for photography Tele 8000 view exposure at turn 2.**
- **Put your RNA sample away in the -80^{o}C freezer**

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• ^ [a] [b] **"*... I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular [..] these pores, or cells, [..] were indeed the first microscopical pores I ever saw, and perhaps, that were ever seen, for I had not met with any Writer or Person, that had made any mention of them before this. . .*" – Hooke describing his observations on a thin slice of cork. Robert Hooke**
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