DNA microarray

- Refer to p. 238 Figure 12.9 for the procedure of DNA microarray

Gel Electrophoresis

- Gel electrophoresis is a technique that uses gel (a thin slab of jellylike material) as a molecular sieve to separate nucleic acids or proteins on the basis of size or electrical charge.
- How gel electrophoresis would be used to separate the various DNA molecules in three different mixtures:
  - A sample of each mixture is placed in a well at one end of a flat, rectangular gel.
  - A negatively charged electrode from a power supply is attached near the DNA-containing end of the gel, and a positive electrode is attached near the other end.
  - Because DNA molecules have negative charge owing to their phosphate groups, they all travel through the gel toward the positive pole.
  - As they move, a thicket of polymer fibers within the gel impedes longer molecules more than it does shorter ones, separating them by length.
  - Thus, gel electrophoresis separates a mixture of linear DNA molecules into bands, each consisting of DNA molecules of the same length, with shorter molecules toward the bottom.

Gel Electrophoresis

- http://learn.genetics.utah.edu/content/labs/gel/

RFLPs

- Unless you have an identical twin, your DNA is different from everyone else's; its total nucleotide sequence is unique.
- Some of your DNA consists of genes, and even more of it is composed of noncoding stretches of DNA.
- Whether a segment of DNA codes for amino acids or not, it is inherited just like any other part of a chromosome. For this reason, geneticists can use any DNA segment that varies from person to person as a genetic marker, a chromosomal landmark whose inheritance can be studied. And just like a gene, a noncoding segment of DNA is more likely to be an exact match to the comparable segment in a relative than to the segment in an unrelated individual.

RFLPs

- Restriction fragment analysis is a method for detecting differences in nucleotide sequence between homologous samples of DNA, usually from two different individuals.
- In restriction fragment analysis, two of the methods we have discussed are used in succession: DNA fragments produced by restricted enzymes are sorted by gel electrophoresis. ****The number of restriction fragments and their sizes reflect the specific sequence of nucleotides in the starting DNA.*
- The differences in restriction fragments produced in this way are called restriction

RFLPs

- How Restriction Fragments Reflect DNA Sequence
  - For example, if a forensic scientists were trying to identify a match between two DNA samples: one obtained from a crime scene and one obtained from a suspect.
  - To detect the differences between the collections of restriction fragments, we need to separate the restriction fragments in the two mixtures and compare their lengths.
  - We can accomplish these things through gel electrophoresis.
  - Then you can compare the bands, and check the

RFLPs

Figure 3

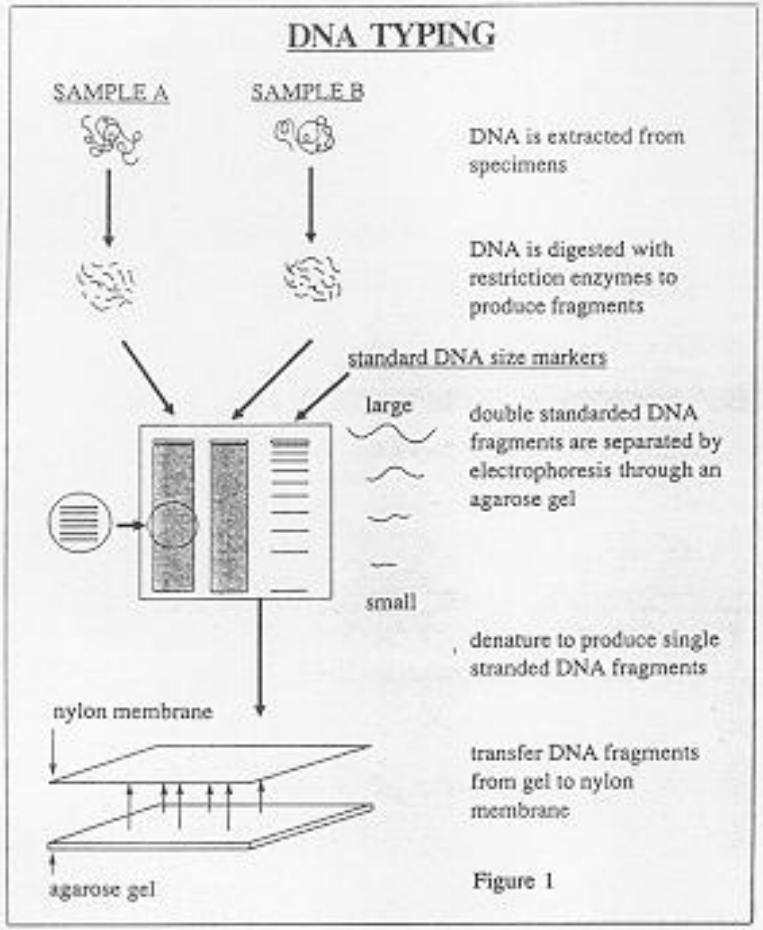


Figure 1

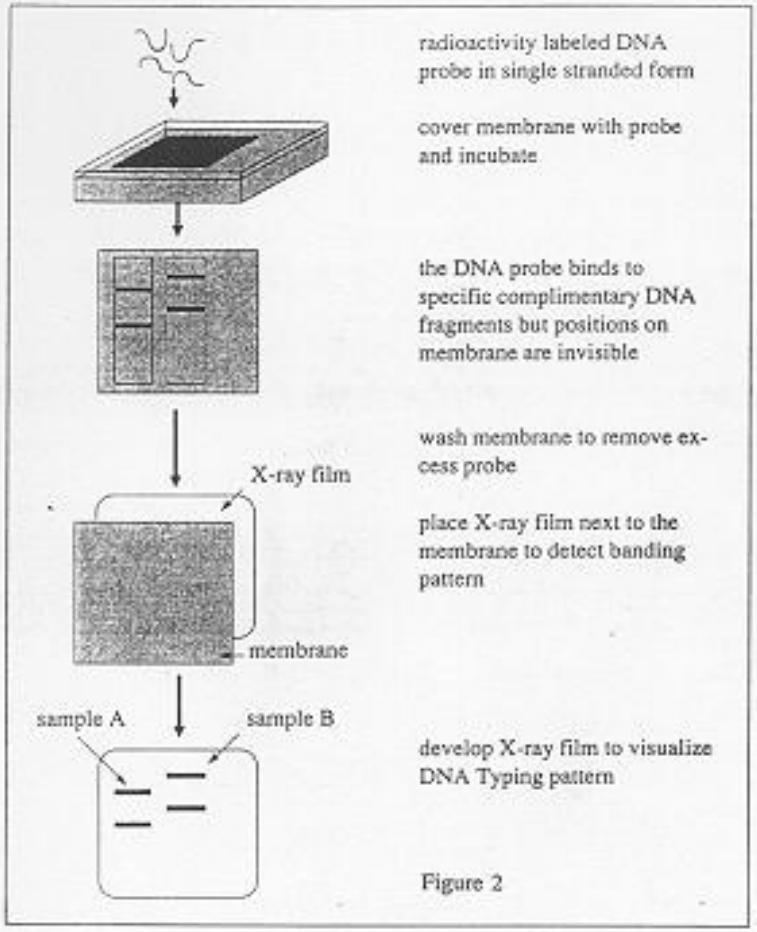


Figure 2

Forensic Science

Forensic Science

- Forensic science is the scientific analysis of evidence for crime scene and other legal investigations, and DNA technology now plays an important role.
- In violent crimes, body fluids or small pieces of tissue may be left at the crime scene or on the clothes of the victim or assailant.
- If rape has occurred, semen may be recovered from the victim's body.
- With enough tissue or semen, forensic scientists can determine the blood type or tissue type using older methods that test for proteins.
- However, such tests require fresh samples in relative large amounts.
- Also, because many people have the same blood or tissue type , this approach can only exclude a suspect; it cannot provide strong evidence of guilt.

Forensic Science

- DNA testing can identify the guilty individual with a high degree of certainty because the DNA sequence of every person is unique (except for identical twins).
- RFLP analysis is one major type of DNA testing .
- It is a powerful method for comparing DNA samples and requires only about 1,000 cells.
- In a murder case, for example, such analysis can be used to compare DNA samples from the suspect, the victim, and bloodstains on the suspect's clothes.
- Radioactive probes mark the electrophoresis bands that contain certain markers.
- Usually about a dozen markers are tested; in other words, only a few selected portions of DNA are compared.
- However, even such a small set of markers from an individual can provide a DNA fingerprint, or specific pattern of bands, that is of forensic use, because the pattern of bands, that is of forensic use, because the probability that two people would have exactly the same set of markers is very small.

Forensic Science

- DNA fingerprinting can also be used to establish family relationships.
  - A comparison of the DNA or a mother, her child, and the purported father can conclusively settle a question of paternity.
  - Sometimes paternity is of historical interest: DNA fingerprinting provide strong evidence that Thomas Jefferson or one of his close male relatives fathered at least one child with his slave Sally Hemings.

Forensic Science

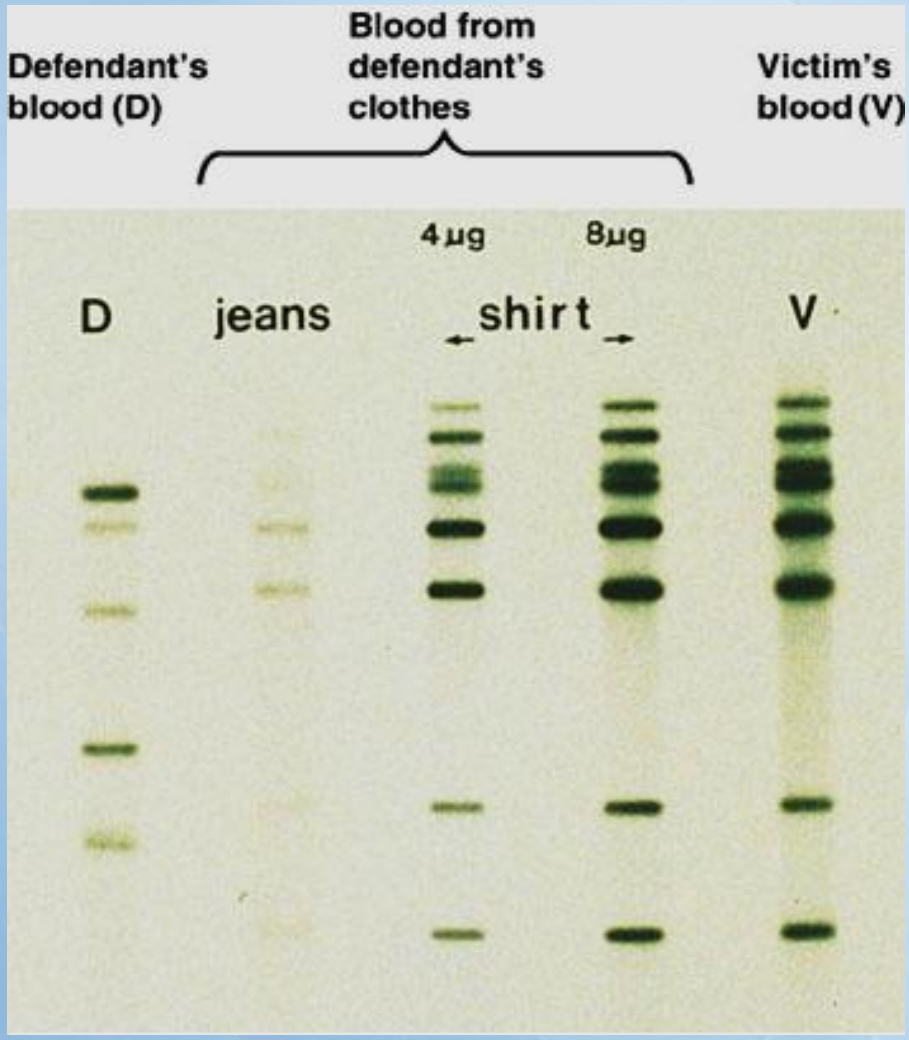
- Today, the markers most often used in DNA fingerprinting are inherited variations in the lengths of repetitive DNA.
  - These repetitive sequences are highly variable from person to person, providing even more markers than RFLPs.
  - For example, one person may have nucleotides ACA repeated 65 times at one genome locus and 118 times at a second locus, whereas another person is likely to have different numbers of repeats at these loci.

Forensic Science

- How reliable is DNA fingerprinting?
  - In most legal cases, the probability of two people having identical DNA fingerprints is between one chance in 10,000 and one in a billion. The exact figure depends on how many markers are in the population. For this reason, DNA fingerprints are now accepted as compelling evident by legal experts and scientists alike.
  - In fact, DNA analysis on stored forensic samples has provided the evidence needed to solve many “cold cases” in recent years. DNA fingerprinting has also exonerated many wrongly convicted people, some of whom were

Forensic Science

DNA Fingerprints From a Murder Case



Forensic Science

- http://www.pbs.org/wgbh/nova/sheppard/analyze.html

Gene Therapy

- Techniques for manipulating DNA have the potential for treating a variety of diseases by gene therapy- alteration of an afflicted individual's genes.
- Theoretically, people with disorders traceable to a single defective gene should be able to replace or supplement the gene with a normal allele.
  - The new allele could be inserted into somatic cells of the tissue affected by the disorder
  - To be permanent, the normal allele would have to be transferred to cells that multiply throughout a person's life

Gene Therapy

- One possible procedure for gene therapy in an individual whose bone marrow cells do not produce a vital protein product because of a defective gene:

1. The normal gene is cloned and then inserted into the nucleic acid of a retrovirus vector that has been rendered harmless.
2. Bone marrow cells are taken from the patient and infected with the virus.
3. the virus inserts its nucleic acid, including the human gene, in the cells' DNA.
4. The engineered cells are then injected back into the patient.

*If the procedure succeeds, the cells will multiply throughout the patient's life and

Gene Therapy

- Although the concept of gene therapy remains promising, very little scientifically strong evidence of effective gene therapy has yet appeared.
- Active research into human gene therapy, with new, tougher safety guidelines, continues.

Gene Therapy

- Human gene therapy raises both techinical and ethical issues.
- <u>Ethical issues:</u>
  - Who will have access to it? The procecures now being tested are expensive and require expertise and equipment found only in major medical centers.
  - Should gene therapy be reserved for treating serious diseases?
  - And, what about its potential use for enhancing athletic ability, physical appearance, and even intelligence?
  - Should we try to eliminate genetic defects in children and their descendants?
- From a biological perspective, the elimination of unwanted alleles from the gene pool could backfire.
- Genetic variation is a necessary ingredient for the survival of a species as environmental conditions change with time.
- Genes that are damaging under some conditions may be advantageous under others (one example is the sickle-cell allele)
- Are we willing to risk making genetic changes that could be detrimental to our species in the future?

Gene Therapy

- Technical issues:
  - How can researchers build in gene control mechanisms to ensure that cells with the transferred gene make appropriate amounts of the gene product at the right time and in the right parts of the body?
  - And how can they be sure that the gene's insertion does not harm some other necessary cell function?

PCR

- DNA cloning in cells is often the best method for preparing large quantities of a particular gene. However, when the source of DNA is scanty or impure, the polymerase chain reaction (PCR) is a much better method.

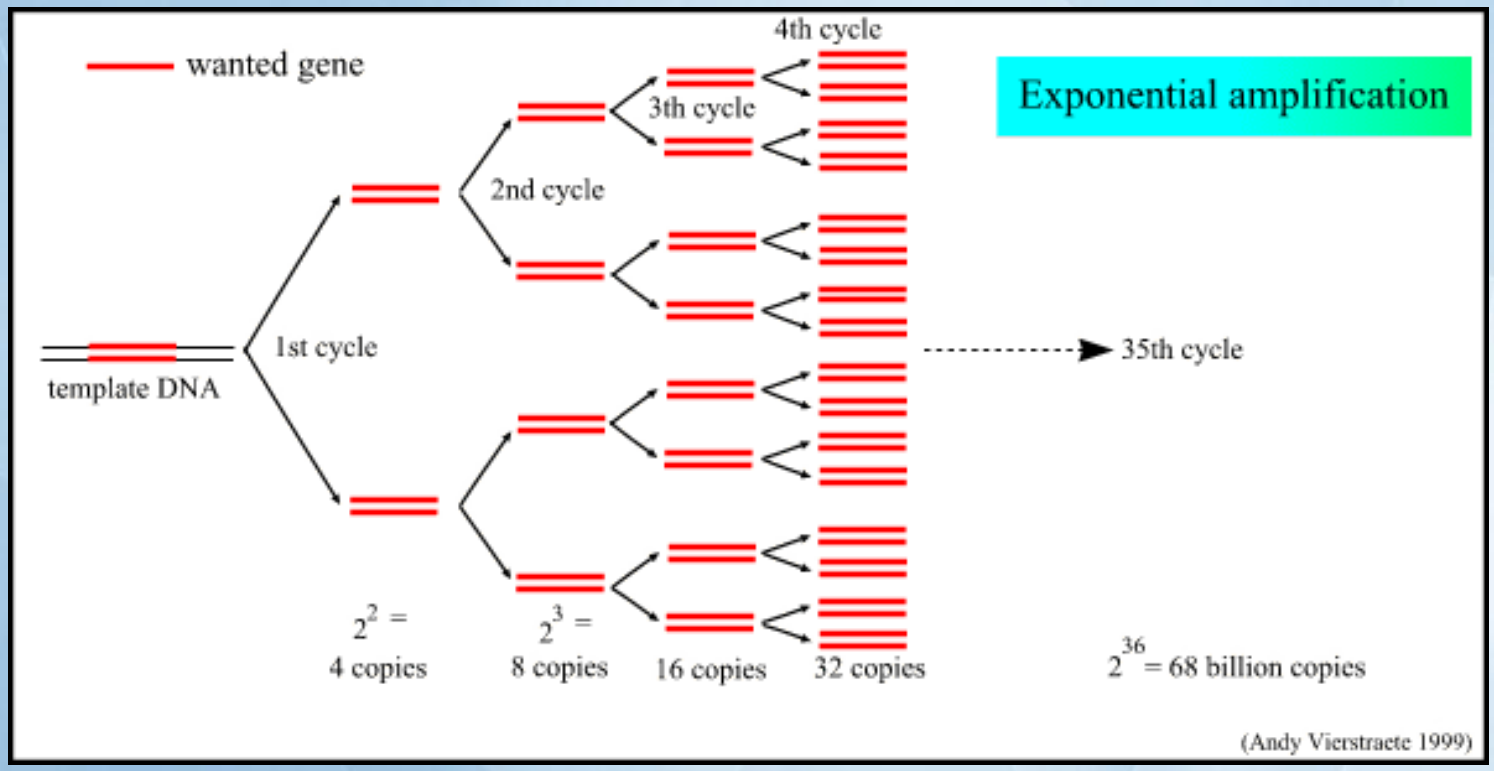
  - In this technique, any specific target segment within a DNA molecule can be quickly amplified (copied many times) in a test tube.

  - Starting with a single DNA molecule, automated PCR can generate 100 billion similar molecules in a few hours.

PCR

- PCR, in principle, is simple.
  - A DNA sample is mixed with the DNA replication enzyme DNA polymerase, nucleotide monomers, and a few other ingredients.
  - The solution is then exposed to cycles of heating (to separate the DNA strands) and cooling.
  - During each cycle, the DNA is replicated, doubling the amount of DNA.
  - For PCR to work, only minute amounts of DNA need be present in the starting material, and this DNA can be in a partially degraded state.
    - From such a scant starting sample, PCR can produce enough DNA for restriction fragment analysis or other DNA technologies.
  - However, occasional errors during PCR replication impose limits on the number of good copies that can be made by this method.
    - So, PCR cannot replace gene cloning in cells when large amounts of DNA are needed.

PCR


wanted gene
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
Exponential amplification
2^2 = 4 copies
2^3 = 8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)

PCR

- Devised in 1985, PCR has had a major impact on biological research and biotechnology.

– It has been used to amplify DNA from a wide variety of sources:

- fragments of ancient DNA from a 40,000 year old frozen woolly mammoth
- DNA from fingerprints or from tiny amounts of blood, tissue, or semen found at crime scenes
- DNA from single embryonic cells for rapid prenatal diagnosis of genetic disorders
- DNA of viral genes from cells infected with such difficult-to-detect viruses such as HIV.

Human Genome Project

- The Human Genome Project (HGP) is an effort to map the human genome in total detail by determining the entire nucleotide sequence of human DNA.
- Begun in 1990, this ambitious project was expected to take 15 years but was largely finished several years ahead of schedule.
- The project was organized by an international, publicly funded consortium of researchers and proceeded through three stages that provided progressively more detailed views of the human genome:

1. Genetic (linkage) mapping –
2. Physical mapping –
3. DNA sequencing –

Human Genome Project

- 1. Genetic (linkage) mapping

– Geneticists combined pedigree analysis of large families with DNA technology to map over 5,000 genetic markers.

– The resulting low-resolution linkage map provided a framework for mapping other markers and for arranging later, more detailed maps of particular regions.

Human Genome Project

- 2. Physical mapping

  - To create a physical map, researchers determined the number of base pairs between markers.
  - This is done by cutting the DNA of each chromosome into a number of restriction fragments, cloning them, and then figuring out the original order of the fragments.
  - The key is to make fragments that overlap and then use probes or automated nucleotide sequencing of the ends to find overlaps. In this way, more and more fragments can be

Human Genome Project

- 3. DNA Sequencing

  – The most arduous part of the project is determining the nucleotide sequences of a set of DNA fragments covering the entire genome, the fragments already mapped in stage 2.

  – Advances in automatic DNA sequencing have been crucial to this endeavor. Sequencing machines can handle DNA molecules up to about 800 nucleotides in length

Human Genome Project

- This three-stage approach is logical and thorough.
- However, in the mid 1990s, J. Craig Venter, a former government scientist, proposed an alternative strategy and set up the company Celera Genomics to implement it.
- Venter's "whole genome shotgun" approach was essentially to proceed directly to the sequencing of small, random DNA fragments, relying on software to determine the order of the pieces.
- Celera actually made significant use of the consortium's data from stages 1 and 2, but the competition between the two groups hastened the progress.
- In February 2001, Celera announced the sequencing of over 90% of the human genome.
- At the same time, HGP researchers made a similar announcement.
- Sequencing of the human genome is now virtually complete, although some gaps remain to be mapped because certain parts of the chromosomes resist mapping by the usual methods.

Human Genome Project

- The potential benefits of having a complete map of the human genome are great:
  - For basic science, the info is already providing insight into such fundamental mysteries as embryonic development and evolution.
  - For human health, the identification of genes will aid in the diagnosis, treatment, and possibly prevention of many of our more common ailments, including heart disease, allergies, diabetes, schizophrenia, alcoholism, Alzheimer's disease, and cancer.
  - Hundreds of disease-associated genes have already been identified as a result of the project.

Human Genome Project

- The DNA sequences from the HGP are deposited in a database available to researchers all over the world via the Internet.
- Scientists use software to analyze the sequences
- Then comes the most exciting challenge: figuring out the functions of the genes and how they work together to direct the structure and function of a living organism.

This challenge and the applications of the new –

Human Genome- not just genes!

- The biggest surprise from the HGP is the small number of human genes. The current estimate is about 20,000 – 25,000 genes, only one and a half to two times the number found in the fruit fly and nematode worm.
- How, then, to account for human complexity?

Part of the answer may lie in alternative RNA – splicing→ scientists think that a typical human gene probably specifies several polypeptides

Human Genome- not just genes!

- In addition to genes, humans, like most complex eukaryotes, have a huge amount of noncoding DNA, about 97% of the total.
  - Some noncoding DNA is made up of gene control sequences such as promoters and enhancers.
  - The remaining DNA includes introns (whose total length may be ten times greater than the exons of a gene) and noncoding DNA located between genes.
  - Much of the DNA between genes consists of

Human Genome- not just genes!

- In one type of repetitive DNA, a unit of just a few nucleotide pairs is repeated many times in a row.
- Stretches of DNA with thousands of such repetitions are prominent at the centromeres and ends of chromosomes, suggesting that this DNA plays a role in chromosome structure.
- Recent research supports the idea that the repetitive DNA at chromosome ends—called telomeres– also have a protective function; a significant loss of telomeric DNA quickly leads to cell death.
- Furthermore, abnormal lengthening of this DNA may help "immortal" cancer cells evade normal

Human Genome—not just genes!

- In the second main type of repetitive DNA, each repeated unit is hundreds of nucleotides long, and the copies are scattered around the genome.
- Most of these sequences seem to be associated with transposons ("jumping genes"), DNA segments that can move or be copied from one location to another in a chromosome and even between chromosomes.
- Transposons can land in the middle of other genes and disrupt them. Reasearchers

Genomics

- Now that sequences of many entire genomes are available, scientists can study whole sets of genes and their interactions, an approach called genomics.
- Genomics is yielding new insights into fundamental questions about genome organization, regulation of gene expression, growth and development, and evolution.

Genomics

- Why map so many genomes?
  - Comparative analysis with the genes of other species also helps scientists interpret the human genome.
  - Also allows us to evaluate the evolutionary relationships between those species.
    - The more similar in sequence, the more closely related those species are by their evolutionary history.

Proteomics

- The success in sequencing genomes and studying whole genomes is encouraging scientists to attempt similar systematic study of the full protein sets (proteomes) encoded by genomes, an approach called proteomics.
- The number of proteins in humans far exceeds the number of genes.
- And since proteins, not genes, actually carry out the activities of the cell, scientists must study when and where proteins are produced in an organism and how they interact in order to understand the functioning of cells and organisms.
- Assembling and analyzing proteomes pose many experimental challenges, but ongoing advances are providing the tools to continue the investigation.

Genomics and Proteomics

- Genomics and proteomics are enabling biologists to approach the study of life from an increasingly global perspective.
- Biologists are now in a position to compile catalogs of genes and proteins—that is, a listing of all the “parts” that contribute to the operation of cells, tissues, and organisms.
- With such catalogs in hand, researchers are shifting their attention from the individual parts to how they function together in biological systems.

Genetically Modified Organisms

- Scientists concerned with feeding the growing human population are using DNA technology to make genetically modified organisms for use in agriculture.
- A GM organism (GMO) is one that has acquire one or more genes by artificial means rather than by traditional breeding methods. (The new gene may or may not be from another species).

Genetically Modified Organisms

- To make genetically modified plants, researchers can manipulate the DNA of a single somatic cell and then grow a plant with a new trait from the engineered cell.
  - Already in commercial use are a number of crop plants carrying new genes for desirable traits, such as delayed ripening and resistance to spoilage and disease.
  - The majority of the American soybean and cotton crops are genetically modified.
  - Many plants have received bacterial genes that make them resistant to herbicides.
  - Health benefits include “Golden rice” which produces grains containing beta-carotene, which our body used to make vitamin A.
    - This could help prevent Vitamin A deficiency—and resulting blindness—among the half of the world’s people who depend on rice as their staple food.

Genetically Modified Organisms

- Agricultural researchers are also making transgenic animals.
  - To do this, scientists first remove egg cells from a female and fertilize them *in vitro*.
  - They then inject a previously cloned gene directly into the nuclei of the fertilized eggs.
  - Some of the cells integrate the foreign DNA into their genomes.
  - The engineered embryos are then surgically implanted in a surrogate mother.
  - If an embryo develops successfully, the result is a transgenic animal, containing a gene from a third “parent” that may even be of another

Genetically Modified Organisms

- Transgenic animals
  - The goal is, for example, to make sheep with better quality wool or a cow that will mature in a shorter time.
  - Scientists might identify and clone a gene that causes the development of larger muscles (which make up most of the meat we eat) in one variety of cattle and transfer it to other cattle or even sheep.
  - Also may be used as pharmaceutical "factories" to produce otherwise rare

Genetically Modified Organisms

- Social concerns:
  - Early concerns focused on the possibility that recombinant DNA technology might create new pathogens.
    - One safety measure is a set of strict laboratory procedures designed to protect researchers from infection by engineered microbes and to prevent the microbes from accidentally leaving the laboratory.
  - Today, most public concern about possible hazards centers not on recombinant microbes but on genetically modified (GM) crops.
    - Advocates of a cautious approach fear that some crops carrying genes from other species might be hazardous to human health or the environment.
    - One specific concern is that genetic engineering

Genetically Modified Organisms

- Today, governments and regulatory agencies throughout the world are grappling with how to facilitate the use of biotechnology in agriculture, industry, and medicine while ensuring that new products and procedures are safe.
- In the US, all projects are evaluated for potentials risks by regulatory agencies such as the FDA, EPA, and NIH, and Department of Agriculture.

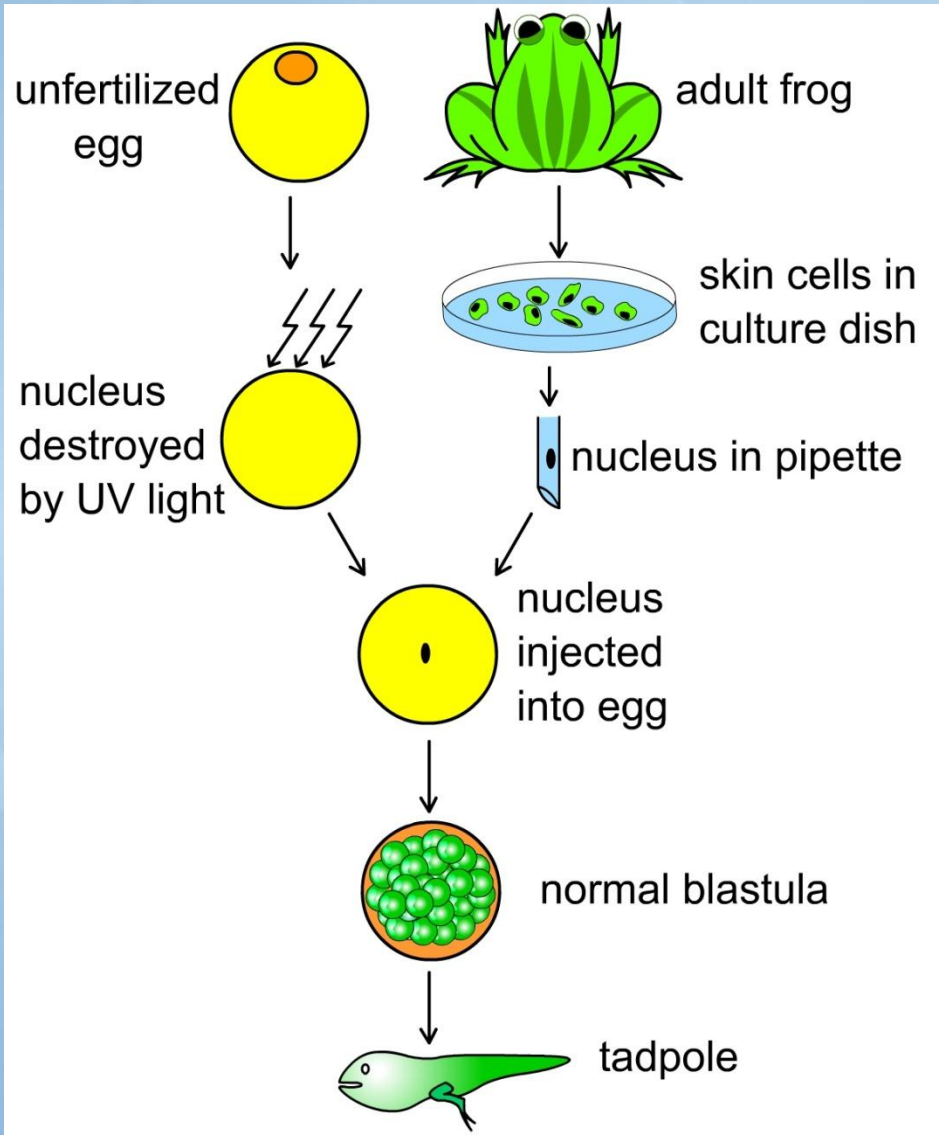
Cloning

Cloning

- Cloning provides strong evidence that differentiated cells retain their full genetic potential.
- Animal cloning is achieved through a procedure called nuclear transplantation.
  - Involves replacing the nucleus of an egg cell or zygote with the nucleus of adult somatic cell.
  - The egg cell may then begin to divide.
  - About 5 days later, repeated cell divisions form a blastocyst, a ball of cells.
  - At this point, the blastocyst may be used for different purposes.

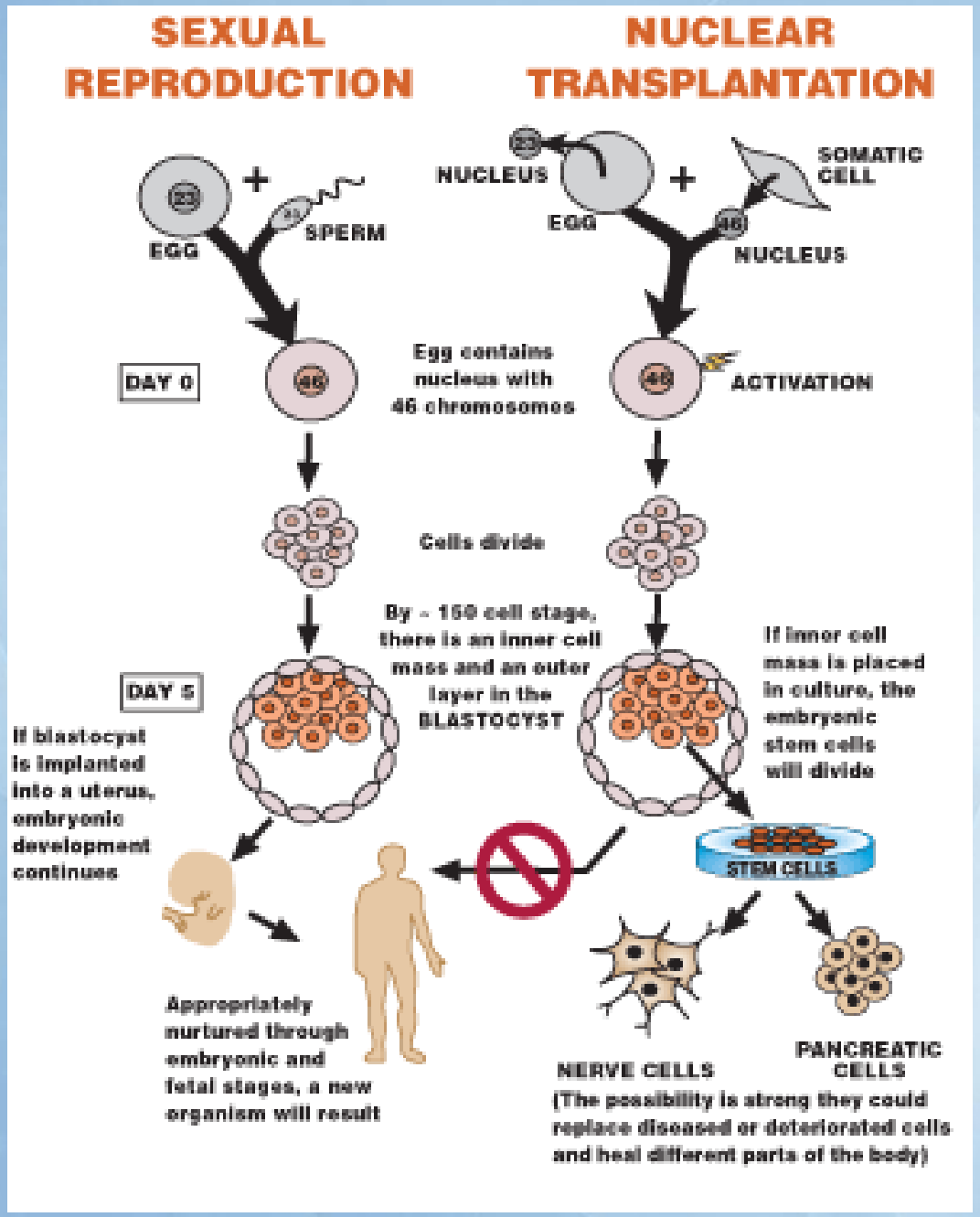
Cloning

- Reproductive cloning
  - If the animal to be cloned is a mammal, further development requires implanting the blastocyst into the uterus of a surrogate mother.
  - The resulting animal will be genetically identical to the donor of the nucleus—a "clone" of the donor.
  - This type of cloning results in the birth of a new individual


unfertilized egg
adult frog
skin cells in culture dish
nucleus destroyed by UV light
nucleus in pipette
nucleus injected into egg
normal blastula
tadpole

Cloning

- Therapeutic cloning
  - Embryonic stem cells (ES cells) are harvested from the blastocyst.
  - In nature, embryonic stem cells give rise to all the different kinds of specialized cells of the body.
  - In the laboratory, embryonic stem cells are easily grown in culture, where, given the right conditions, they can perpetuate themselves indefinitely.

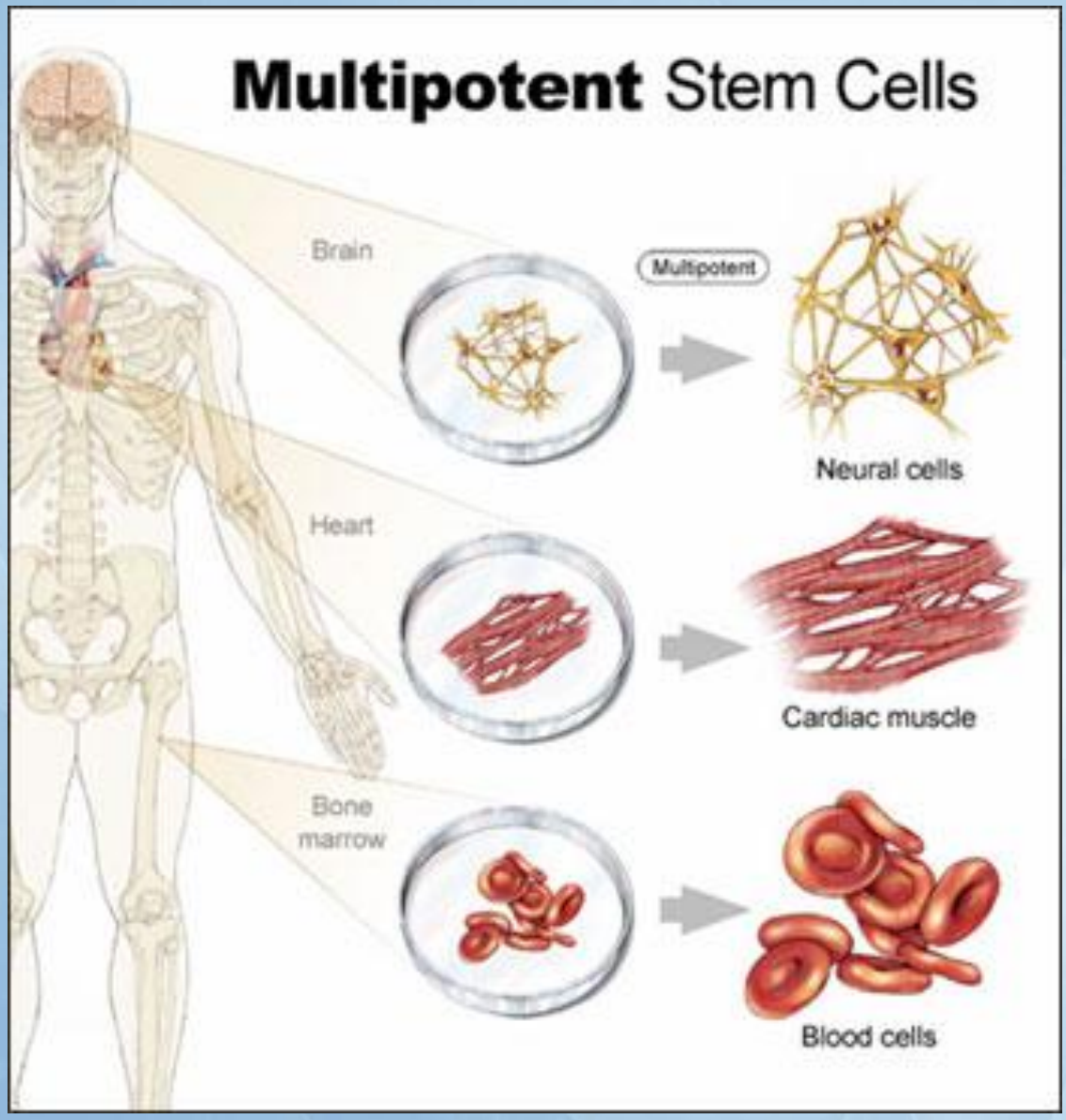

SEXUAL REPRODUCTION
NUCLEAR TRANSPLANTATION
EGG
SPERM
NUCLEUS
SOMATIC CELL
NUCLEUS
DAY 0
Egg contains nucleus with 46 chromosomes
ACTIVATION
Cells divide
By ~ 150 cell stage, there is an inner cell mass and an outer layer in the BLASTOCYST
If inner cell mass is placed in culture, the embryonic stem cells will divide
DAY 5
If blastocyst is implanted into a uterus, embryonic development continues
STEM CELLS
Appropriately nurtured through embryonic and fetal stages, a new organism will result
NERVE CELLS
PANCREATIC CELLS
(The possibility is strong they could replace diseased or deteriorated cells and heal different parts of the body)

Cloning

- Therapeutic cloning applications:
  - Therapeutic cloning produces ES cells that in the early animal embryo differentiate to give rise to all the cell types in the body.
  - When grown in laboratory culture, ES cells can divide indefinitely (like cancer cells)
  - But the right conditions—such as the presence of certain growth factors—can induce changes in gene expression that cause differentiation into a particular cell type.
  - If scientists can discover the right conditions, they will be able to grow cells for the repair of injured or diseased organs.
  - Such cells could be made by inserting a cell nucleus from a patient into an ES cell from which the nucleus has been removed.
  - When implanted in the patient, these cells would not be rejected by the immune system because they would be genetically identical to the patient's own cells.

Cloning

- ES cells raise both ethical and technical problems.
  - Human ES cells must be obtained by destroying human embryos (such as ones donated by patients undergoing infertility treatment).
  - This might be avoided by using adult stem cells, cells present in adult tissues that generate replacements for nondividing differentiated cells.
- Unlike ES cells, adult stem cells are part way along the road to differentiation.
- They can often give rise to multiple types of specialized cells, but it is not clear whether they can give rise to *all* types of cells.
- Like ES cells, adult stem cells can be grown in culture and induced to differentiate into a range of cell types.
- For example, adult stem cells in bone marrow generate all types of blood cells.
- Perhaps adult stem cells, ethically less problematic to obtain than ES cells, may provide the answer to human tissue and organ replacement.
- However, ES cells are currently more promising than adult stem cells.


Multipotent Stem Cells
Brain
Multipotent
Neural cells
Heart
Cardiac muscle
Bone marrow
Blood cells