

OVERVIEW OF BIOTECHNOLOGY

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Section 1

BACKGROUND

OVERVIEW

THE U.S. DEPARTMENT OF AGRICULTURE (USDA)'S CLASSICAL INTERPRETATION

Agricultural biotechnology is a collection of scientific techniques, including genetic engineering, that are used to create, improve, or modify plants, animals, and microorganisms...

- Agricultural Research Service (ARS), the in-house research agency of USDA, classifies biotechnology research into six components:
 - basic engineering of recombinant DNA;
 - DNA sequencing;
 - genomic mapping with molecular markers;
 - monoclonal antibodies;
 - cell fusion and chromosome transfer;
 - biologically-based processing

CLASSICAL PLANT BREEDING

- Practicing classical plant breeding means many thousands of plants must be cross-pollinated to find the one offspring with higher yield.
- In crossing plants,
 - Pollen must be taken from one plant and manually placed on another.
 - The possibility of finding improved traits is limited by the amount of genetic diversity already present in the plants.
 - Consequently, if the two plants that are crossed share many of the same genes, the amount of possible improvement is limited.
- Therefore, scientists have searched for better ways to improve plants.

MUTATION BREEDING

- In the 1920s, scientists realized that *mutations* could be induced in seeds by using chemical mutagens or by exposure to X-rays or gamma rays.
- Outcome of such treatments is even less predictable than traditional breeding methods.
- Successful in world of flowers; new colors and more petals have been expressed in flowers such as tulips, snapdragons, roses, chrysanthemums, and many others.
- Mutation breeding has also been tried on vegetables, fruits, and crops. For instance, peppermint plants that are resistant to fungus were generated this way.



TRANSGENIC TECHNOLOGY VS TRADITIONAL BREEDING

- A plant can be transformed with a gene from any source, including animals, bacteria, or viruses as well as other plants, whereas traditional cross-breeding methods move genes only between members of a particular genus of plants.
- Furthermore, transgenes can be placed in precise locations within the genome and have known functions that have been evaluated extensively before being inserted into the plant.
- In traditional breeding, on the other hand, the identity of genes responsible for improving the crop is rarely known.
- Introduction of molecular breeding – more predictable way to enhance crops.
- Movement of genes from foreign sources into a specific plant, resulting in a *transgenic* plant.
- The foreign gene, or transgene, may confer specific resistance to an insect, protect the plant against a specific herbicide, or enhance the vitamin content of the crop.
- With something so powerful as genetic engineering, one mistake could have profound and wide-ranging effects. We must impose tough controls on the genetics supply industry and work to make sure that the new techniques are in the service of the global community

Section 2

HISTORY

HISTORY

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TIMELINE

TABLE 1: History of biotechnology

Date	Event
5000 BC	Indus and Indo-Aryan civilizations practiced biotechnology to produce fermented foods and medicines and to keep the environment clean.
4000 BC	Egyptians used yeasts to make wine and bread.
1750 BC	The Sumerians brewed beer.
250 BC	The Greeks used crop rotation to maximize crop fertility.
1500 AD	The Aztecs made cake from spirulina.
1663 AD	Robert Hook first described cells.
1675 AD	Microbes were first described by Anton Van Leeuwenhook.
1859 AD	Darwin published his theory of evolution in 'The Origin of Species.'
1866 AD	Gregor John Mendel published the basic laws of genetics.
1869 AD	DNA was isolated by Friederich Miescher.
1910 AD	Genes were discovered to be present in chromosomes.
1917 AD	The term 'biotechnology' was used to describe fermentation technology.
1928 AD	The first antibiotic, penicillin, was discovered by Alexander Flemming.
1941 AD	The term 'genetic engineering' was first used.
1944 AD	Hereditary material was identified as DNA.
1953 AD	Watson and Crick proposed the double helix structure of DNA.

TIMELINE

TABLE 2: History of biotechnology (...continued)

Date	Event
1961 AD	Deciphering of genetic code by M.Nirenberg and H.G. Khorana.
1969 AD	The first gene was isolated.
1973 AD	The first genetic engineering experiment was carried out by Walter Gilbert.
1975 AD	Creation of the first hybridomas.
1976 AD	The first biotech company.
1978 AD	World's first 'test-tube baby,' Louise Brown, was born through in vitro fertilization.
1981 AD	The first gene was synthesized. The first DNA synthesizer was developed.
1982 AD	The first genetically engineered drug, human insulin, produced by bacteria, was manufactured and marketed by a U.S. company. Production of the first monoclonal antibodies for diagnostics.
1983 AD	The first transgenic plant was created—a petunia plant was genetically engineered to be resistant to kanamycin, an antibiotic.
1983 AD	The chromosomal location of the gene responsible for the genetic disorder, Huntington's disease, was discovered leading to the development of genetic screening test.
1985 AD	DNA fingerprinting was first used in a criminal investigation.
1986 AD	The first field tests of genetically-engineered plants (tobacco) were conducted.
1990 AD	Chymosin, an enzyme used in cheese making, became the first product of genetic engineering to be introduced into the food supply
1990 AD	Human genome project was launched.
1990 AD	The first human gene therapy trial was performed on a four-year-old girl with an immune disorder.
1991 AD	The gene implicated in the inherited form of breast cancer was discovered

TIMELINE

TABLE 3: History of biotechnology (...continued)

Date	Event
1992 AD	Techniques for testing embryos for inherited diseases were developed
1994 AD	First commercial approval for transgenic plant by the U.S. government.
1995 AD	First successful xenotransplantation trial was conducted, transplanting a heart from a genetically-engineered pig into a baboon.
1996 AD	First commercial introduction of a 'gene chip' designed to rapidly detect variances in the HIV virus and select the best drug treatment for patients.
1996 AD	Dolly, the sheep was cloned from a cell of an adult sheep.
1998 AD	Embryonic stem cells were grown successfully, opening new doors to cell- or tissue-based therapies.
1999 AD	A U.S. company announced the successful cloning of human embryonic cells from an adult skin cell.
1999 AD	Chinese scientists cloned a giant panda embryo.
1999 AD	Indian scientists and companies started producing recombinant vaccines, hormones, and other drugs.
1999 AD	The draft of human genome sequence was published.

Section 3

MAJOR TECHNIQUES AND APPLICATIONS OF BIOTECHNOLOGY

CELLULAR TECHNIQUES

- Microscopy
- Cell sorting
- Cell fractionation
- Cell-growth determination

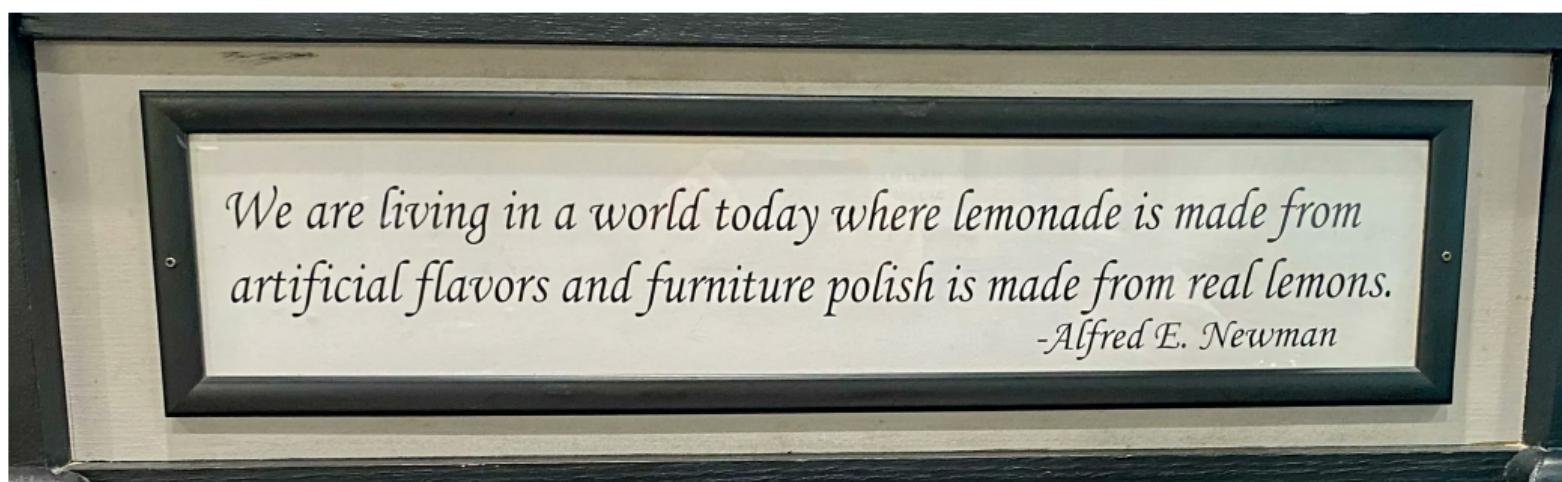
GENETIC TECHNIQUES

- Chromosomal techniques
- Mutagenic technique
- Recombination in bacteria (Recombination DNA technology)
 - Tools
 - Making Recombinant DNA
 - DNA library
 - Transgenics (Introduction of Recombinant DNA into host cells)
 - Identification of recombinants
 - Polymerase chain reaction
 - DNA probes
 - Hybridization techniques
 - DNA sequencing
 - Site-directed mutagenesis
- Pedigree analysis in humans
- DNA isolation and purification techniques
- Molecular markers, TILLING and ZFN technology in plants

MAJOR APPLICATIONS OF BIOTECHNOLOGY

- Biological fuel generation
- Single-cell protein
- Sewage treatment
- Environmental biotechnology
- Medical biotechnology
- Agriculture and forest biotechnology
- Food and beverage biotechnology
- Safety in biotechnology

MAJOR CRITICISMS AGAINST BIOTECHNOLOGY

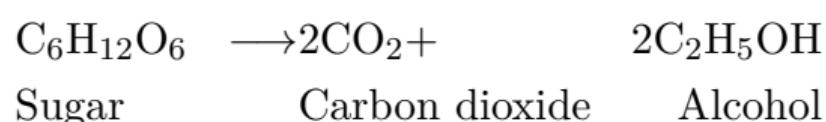


Section 4

BIOTECHNOLOGY TYPES AND PROCESSES

FERMENTATION

- Decomposition of foodstuffs generally accompanied by the evolution of gas.
- The best-known example is alcoholic fermentation, in which sugar is converted into alcohol and carbon dioxide.
- This conversion, described by the equation below, was established by J. L. Gay-Lussac in 1815.



HISTORY

- Before 1800 the association of yeast or leaven with fermentation had been noted, but the nature of these agents was not understood.
- Experiments of C. Cagniard-Latour, of F. T. Kutzin, and of T Schwann in 1837 indicated that yeast is a living organism and is the cause of fermentation.
- This view was opposed by such leading chemists as J. von Liebig and F. Wohler, who sought a chemical rather than a biological explanation of the process.
- The biological concept became generally accepted following the work of Louis Pasteur, who concluded that fermentation is a physiological counterpart of oxidation, and permits organisms to live and grow in the absence of air (anaerobically).

HISTORY

- This linked fermentation and putrefaction as comparable processes; both represent decompositions of organic matter brought about by microorganisms in the absence of air.
- The difference is determined by the nature of the decomposable material;
 - sugary substances generally yield products with pleasant odor and taste (fermentation),
 - proteins give rise to evil-smelling products (putrefaction).
- Pasteur also discovered the lactic acid and butyric acid fermentations, and from his experiments concluded that each kind of fermentation was caused by a specific microbe.
- Later work supported this idea to a large extent, and considerably increased the number of specific fermentations.

PROCESS

During fermentation organic matter is decomposed in the absence of air (oxygen); hence, there is always an accumulation of reduction products, or incomplete oxidation products. Some of these products (for example, alcohol and lactic acid) are of importance to society, and fermentation has therefore been used for their manufacture on an industrial scale. With regard to historic roots of the process, Converting dry grains and other seeds into something more appetizing than a gruel must have made agriculture more attractive and valuable. Alcohol, despite its dangers, provided (and still provides), in reasonable moderation, a basis for social interaction. There are also many microbiological processes that go on in the presence of air while yielding incomplete oxidation products. Good examples are the formation of acetic acid (vinegar) from alcohol by vinegar bacteria, and of citric acid from ugar by certain molds (for example, *Aspergillus niger*). These microbial processes, too, have gained industrial importance, and are often referred to as fermentations, even though they do not conform to Pasteur's concept of fermentation as a decomposition in the absence of air.

FERMENTATION TECHNOLOGY: MILK FERMENTATION

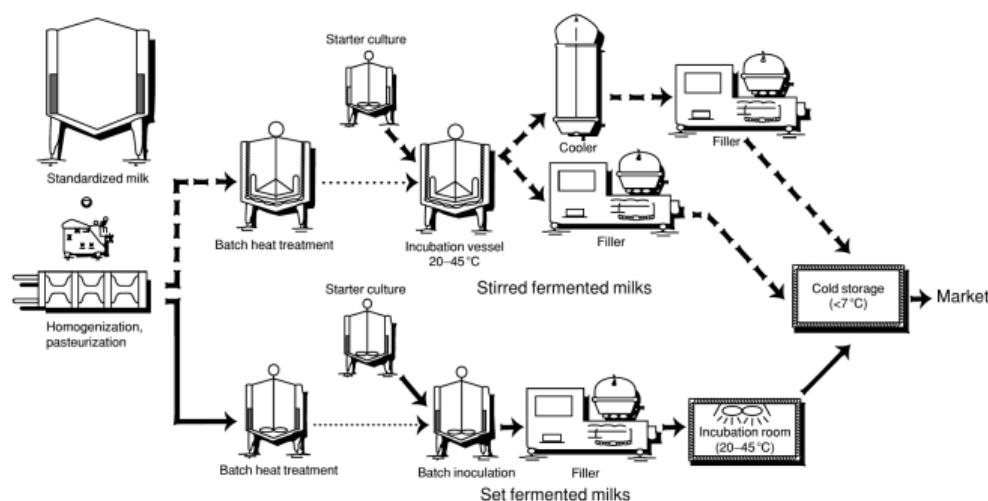


FIGURE 1: Basic steps in manufacture of fermented milks. From International Dairy Federation (1988) Fermented Milks-Science and Technology. International Dairy Federation Bulletin No. 227.

BIOTECHNOLOGY PROCESS (OVERVIEW)

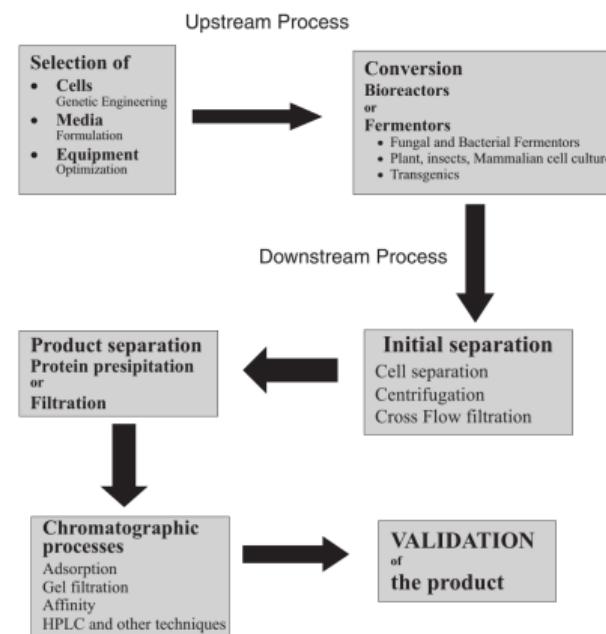


FIGURE 2: A typical biotechnology flow process

INDUSTRIAL BIOTECHNOLOGY

- Recombinant microorganisms, plant cells, and animal cells can be cultivated and used for large-scale production of industrially-important enzymes and chemicals. A list of such enzymes is given in Table 4.

TABLE 4: Some major industrial enzymes and their sources and uses.

Enzymes	Sources	Uses
Amylases	Aspergillus niger, A. oryzae, B. licheniformis, B. subtilis, germinating cereals germinating barley	Hydrolyze starch to glucose, detergents, baked goods, milk cheese, fruit juice, digestive medicines, dental care
Invertases	Saccharomyces cerevisiae	Production of invert sugar, confectionery
Glucose isomerase	Arthrobacter globiformis, Actinoplanes missouriensis, Streptomyces solivaceus and E. coli	Conversion of glucose to fructose production of high fructose syrup, other beverages, and food
α D-Galactosidase	Mortierella vinaceae	Raffinose hydrolysis
β D-Galactosidase	Aspergillus niger	Lactose hydrolysis
Papain	Papaya	Meat, beer, leather, textiles, pharmaceuticals, meat industry, digestive aid, dental hygiene, etc.
Proteases	Bacillus subtilis, B. licheniformis	Detergents, meat tenderizers, beer, cheese, flavor production
Pepsin	Hog (pig) stomachs	Cereals, pharmaceuticals
Trypsin	Hog and calf pancreases	Meat, pharmaceuticals
11-β-Hydroxylase	Curvularia lunata	Steroid conversion, bioconversion of organic chemicals
Ficin	Figs	Leather, meat, pharmaceuticals
Bromelain	Pineapple	Meat, beer, pharmaceuticals

Section 5

ENVIRONMENTAL BIOTECHNOLOGY

BACKGROUND

- People have always been fascinated by the environment around them and successful in harnessing the environment for our benefit.
- Curiosity to explore uncharted life forms drives our motivations.
- Virosphere! How big is it?
- Human body harbors 10^{14} bacteria, while our own only comprise 10^{13} .

APPLICATIONS

- An increase in the productivity of crops, without an increase in the dependency on environmentally-damaging agrochemicals.
- As a result of increased productivity, a reduced pressure to exploit the remaining uncultivated habitats.
- As a result of increased productivity, a reduction in energy inputs (mostly from reduced agrochemical manufacture).
- The creation of alternative, renewable, sources of energy (e.g., biodiesel).
- The creation of new more environment-friendly raw materials for industry (e.g., biodegradable plastics from plant starches, or high-value speciality chemicals).
- As a result of the development of genetically-modified crops (if properly used), a reduction in the amount of agrochemical (e.g., pesticides and herbicides) released into the environment.

BIO-ENVIRONMENTAL PROCESSES

Bioremediation

- One of the avenues in biotechnology that has made rapid advances
- “biological” means of cleaning the environment.
- Naturally occurring microorganisms often have the ability to degrade human-made pollutants.
- *Rhodococcus* sp. has a highly diverse pathways to degrade pollutants, such as short- and long-chain alkanes, aromatic molecules (both halogenated and nitro-substituted), and heterocyclic and polycyclic aromatic compounds, including quinolone, pyridine, thiocarbamate, s-triazine herbicides, 2-mercaptobenzothiazole (a rubber vulcanization accelerator), benzothiophene, dibenzothiophene, MTBE, and the related ethyl tert-butyl ether (ETBE).

BIO-ENVIRONMENTAL PROCESSES

- **Biostimulation** is the release of nutrients, oxidants, or electron donors into the environment to stimulate naturally occurring microorganisms to degrade a contaminant.
- **Bioaugmentation** is adding specific microorganisms plus their energy sources to decontaminate a polluted area.
- **Microbial fuel cells** create electricity through the use of microorganisms. Organisms that transfer electrons to the anode are called electrode-reducing organisms. They can pass electrons through a mediator molecule in the solution, directly through proteins in their outer membrane, or through nanowires or pili that coat the outer surface of the bacterium. Electrode-oxidizing organisms take electrons from the cathode to reduce various substances, such as carbon dioxide to acetate.

BIOFUEL PRODUCTION

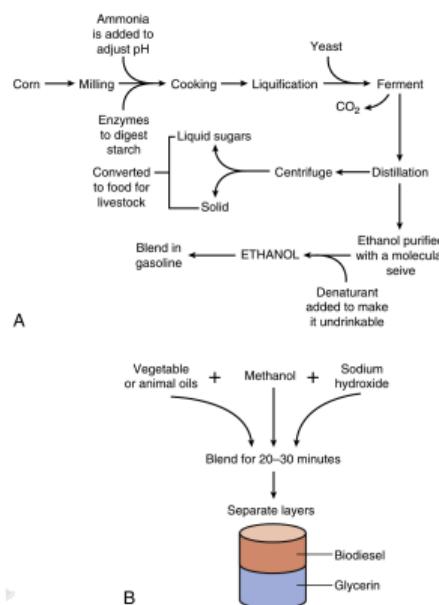


FIGURE 3: (A) Production of ethanol from corn requires the addition of ammonia to adjust the pH and enzymes to help digest starch and yeast to ferment the corn mash. (B) Biodiesel is created by the blending of methanol, sodium hydroxide, and vegetable oil for 20-30 minutes.

MOLECULAR GENETICS AND PROTEIN DETECTION

- PCR is routinely used to amplify random sequences from many environmental samples in the hope of identifying new genes.
- After PCR DNA is sequenced.
- Then bioinformatics reveals whether or not the sequence (or a close relative) has already been identified or if it is completely novel.
- Microarrays are used to compare numbers and types of organisms present in different environment.

IDENTIFYING NEW GENES WITH METAGENOMICS

- Allows identification of microorganisms, viruses, or free DNA that exist in the natural environment.
- Approaches: next-generation DNA sequencing, PCR, RT-PCR and microarrays
- Metagenomics is the process of statistically combining separate genomic analyses; deals with a mixture of DNA forms.
- Study of marine microbiology, human gut microbiology, assessment of how microorganisms form symbiotic relationships with their hosts, finding novel antibiotics or enzymes, replacement of chemical pesticides with crops genetically engineered for tolerance to microorganisms and nematodes, etc.

CURRENT CONCERNS AND SOLUTIONS

Concerns

- Herbicide use
- Genetic pollution and superweeds
- Antibiotic resistance
- Unexpected effects
- Pest resistance
- Persistence and weediness
- Damage to wildlife and biodiversity

Avenues

- Genetic modification of plants achieves essentially the same result as conventional plant breeding
- Wide-crossing
- In Nature, genes are exchanged between species
- Gene instability in conventional crops
- Conventional breeding is not 'Natural' either
- Gene transfer from crops to their 'Wild' relatives
- Novel, sustainable, agricultural practices needed

STUDY TECHNIQUES

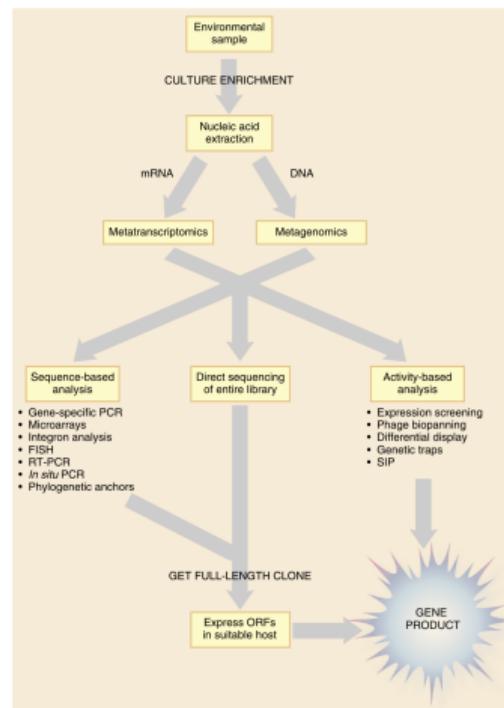


FIGURE 4: Techniques to study environmental samples

Section 6

MEDICAL BIOTECHNOLOGY

APPLICATIONS

- Cheaper medicines from biotechnology
- Medicines from 'cultured' cells
- Genetic modification for medicine production
- Immune technology; Killed pathogens, attenuated pathogens, single proteins, or epitopes from a disease-causing pathogen are used as vaccines. They are isolated and injected into people to elicit their immune response without causing the disease. Multivalent vaccines contain antigens to different proteins from a pathogen or family of pathogens.
- Cancer technology; Oncogene detection, oncogene attenuation

POPULAR TECHNOLOGIES

- Gene therapy; Engineered retroviruses are the most frequently used viral vectors in gene therapy. Defective retrovirus vectors are grown in cells with an integrated helper virus to allow formation of virus particles.
- ELISA assay; Antibodies are used in ELISA assays to determine the relative concentration of the target protein or antigen in a sample. Primary antibodies recognize the target protein or antigen. Secondary antibodies recognize the primary antibody and often carry a detection system. Secondary antibodies are made to recognize any antibody that is made in sheep, cow, rabbit, goat, or mouse.

STEM CELL THERAPY

- Characteristics of stem cells:
 - they maintain the ability to divide continually,
 - they are undifferentiated, and
 - they have the ability to differentiate into multiple cell types
- Embryonic stem cells are totipotent.
- Adult or somatic stem cells are able to differentiate into different cell types but are multipotent; that is, they are restricted to the tissues in which they originate.
- Embryonic stem cell lines are created from the inner cell mass of the blastula stage of an embryo from many different mammals, including humans, mice, and primates.
- The cell lines can be induced to differentiate by forming embryoid bodies that ultimately differentiate into different cell types.

STEM CELL THERAPY

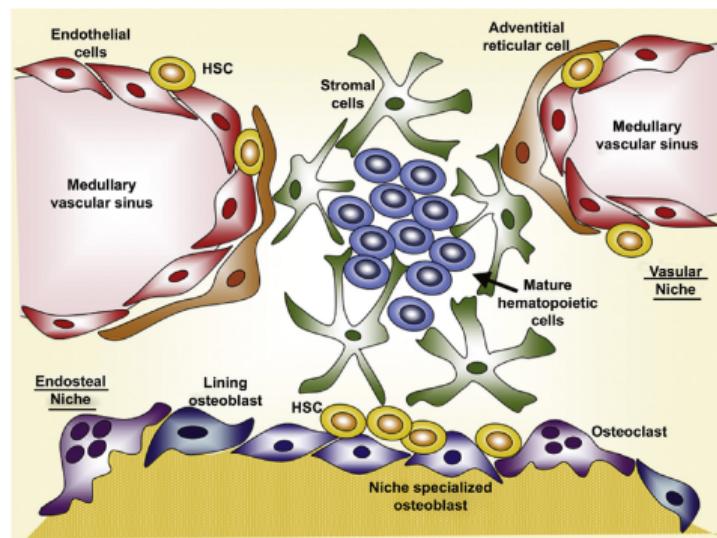


FIGURE 5: Hematopoietic stem cells are located near the interface of the bone marrow and bone surface (the endosteal niche) and also near vascular sinuses (vascular niche). The HSCs in each niche divide to form mature hematopoietic cells that populate the marrow tissues.

CLONING DOLLY

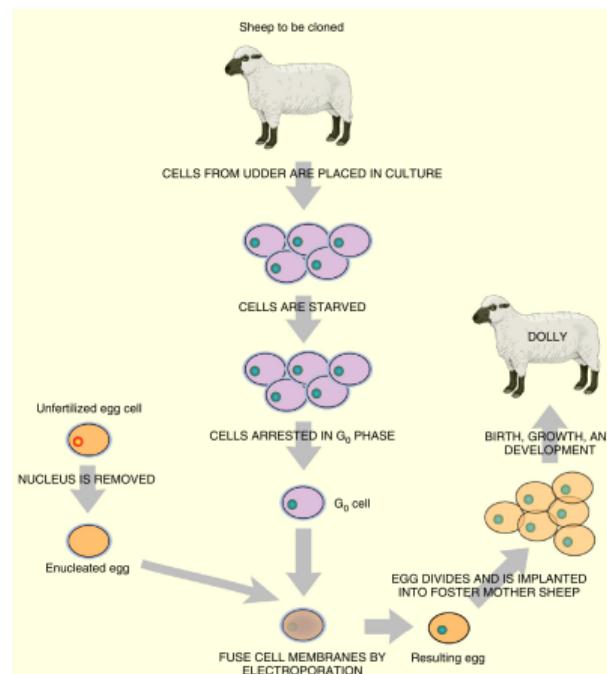


FIGURE 6: To clone a mammal such as a sheep, cells from the udder are isolated, grown in culture, and then starved in order to arrest them in G₀ of the cell cycle. Unfertilized egg cells from another sheep are also harvested, and the nucleus is removed. An electrical stimulus fuses the G₀ udder cell with the enucleated egg, thus placing a somatic cell nucleus into an undifferentiated cytoplasm. The eggs that result are put back into a foster mother, and the offspring are screened for DNA identical to the donor sheep.

Section 7

FORENSIC BIOTECHNOLOGY

- Researches and uses the basis of identity to resolve conflicting situations and help create unique database of individuals.
- Fingerprint patterns are multigenic trait.
- Retinal scans take advantage of the unique pattern of blood vessels on the retina at the back of the eyes.
- Blood typing provides identity based on presence of blood antigens groups.

DNA FINGERPRINTING

- DNA fingerprinting relies on the unique pattern made by a series of DNA fragments after separating them according to length by gel electrophoresis.
- The samples are then processed to generate a set of DNA fragments. When Alec Jeffreys invented DNA fingerprinting in 1985 in England, the DNA was cut with restriction enzymes to generate fragments because PCR had not yet been invented.
- Nowadays, DNA is prepared by PCR, and fluorescent dyes are used for labeling. In addition, modern DNA fingerprinting uses repeated sequences (short tandem repeats or STRs) for routine identification purposes.

DNA FINGERPRINTING

- For the first generation of DNA fingerprints, restriction enzymes were used to generate the variation in DNA fragment size between individuals. Variations in the DNA base sequence of restriction enzyme recognition sites result in differences in the size of the fragments.
- Such sequence differences are called restriction fragment length polymorphisms (**RFLPs**).
- Many different restriction enzymes with distinct recognition sites are used on each DNA sample.
- Even if mutations have changed a few bases of the target sequence around the cut site, there is usually still enough similarity for probes to bind.

RFLP STEPS

- The DNA is cut with a restriction enzyme.
- The DNA fragments are separated by length or molecular weight by gel electrophoresis.
- The fragments are visualized by Southern blotting. The separated fragments are transferred from the gel to nylon paper. Then a radioactively labeled DNA probe is added.
- The probe binds to those DNA fragments with complementary sequences.
- The blot is covered with radiation-sensitive film to give an autoradiograph. This shows the location of those DNA fragments that reacted with the radioactive probe.
- The final product of a DNA fingerprint is an autoradiograph that contains at least five essential lanes (Figure 8). The markers are standardized DNA fragments of known size, which have been radioactively labeled.

RFLP FINGERPRINTING

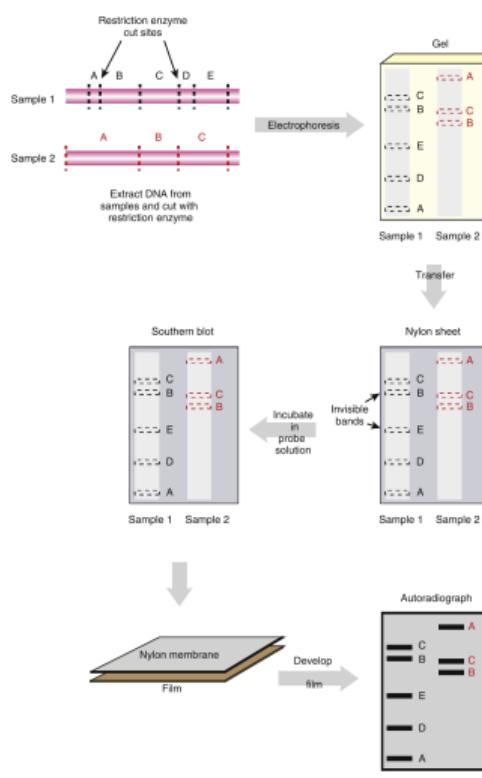


FIGURE 7: Outline of RFLP based DNA fingerprinting

RFLP FINGERPRINTING

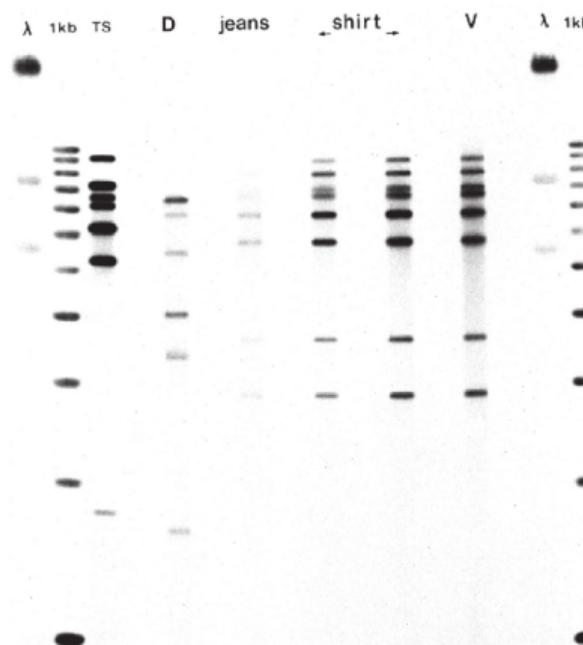


FIGURE 8: Actual DNA fingerprint showing that the pattern of DNA fragments of the victim (V) were found on the defendant's clothing (jeans/shirt). The first two and last two lanes are the standard size markers (labeled λ and 1 kb). The lane marked TS is a positive control showing that the fingerprint technique was successful. The lane marked D is the defendant's DNA pattern.

Section 8

BIBLIOGRAPHY

FURTHER STUDY

Also see: Nair ([2008](#))

REFERENCES

Nair, A Jayakumaran. 2008. *Introduction to Biotechnology and Genetic Engineering*. Laxmi Publications, Ltd.