Agriculture includes all the practices of growing and harvesting crops, including soil management, water management, plant and animal breeding and hybridization, asexual plant propagation, seed production and improvements and crop management. It also includes the use of fertilizers, herbicides, insecticides, pesticides and the improvement of farming equipment. New biotechnology techniques have been applied to improve the quantity and quality of agriculture products including genetic testing, plant tissue culture, DNA manipulation, gene transfer, protein manipulations, genetic engineering and plant/animal cloning.

Agriculture biotechnology is a range of tools which alter living organisms or parts of organisms to make or modify products, improve plants or animals, or develop microorganisms for specific agriculture uses.

- Using gene transfer technologies, biotechnologists can add or modify specific genes to produce different proteins and offspring with predictable, desired traits with less risk of unwanted characteristics form a wide range of donor organisms.
- Using gene modification, agriculturists have developed plants that can be grown with fewer applications of chemicals or pesticides. Example: Bt crops are resistant to several pests. The Bt delta endotoxin protein was originally made form *Bacillus thuringiensis*. By inserting the Bt gene from bacteria into plants, the plants become protected from insect damage.
- Selective breeding, DNA fingerprinting and gene introduction into livestock and plants have improved nutritional value, increased growth rate, or require less fertilizers.

**Research:**

Use this site to answer the questions. [http://cls.casa.colostate.edu/TransgenicCrops/faqpopup.html](http://cls.casa.colostate.edu/TransgenicCrops/faqpopup.html)

1. Why do we want to make transgenic crops?
2. What genetically engineered crops are actually being grown now?
3. Are there really economic benefits for farmers growing transgenic crops? Explain.
4. Has there actually been a reduction in pesticide use resulting from planting transgenic crops? Explain.
5. How do the few kinds of GE crops end up in such a high percentage of our food?
6. How do we know genetically engineered crops are safe to eat?
7. Why is there so much debate about mandatory labeling of genetically engineered foods?
8. How is "substantial equivalence" used to determine the safety of genetically engineered food?
9. Why do GE plants have antibiotic resistance genes? Doesn't this pose a risk for developing resistant strains of bacteria?
10. I'm concerned by the reports of transgenic DNA found in Mexican corn landraces. Isn't this evidence that transgenic crops will cause environmental damage by reducing genetic diversity?

**Advances in Agriculture through DNA Technology**

Once bacteria cells have been genetically modified, scientists attempted similar techniques to introduce foreign DNA into plants to modify their phenotypes. Agricultural scientists began developing the processes necessary to genetically engineer agriculture products.

1) Isolating plant genomic DNA (gDNA): To isolate the gDNA in most cells, cell lysis buffer is used to burst open the cell. A protein-degrading buffer is used to denature the protein contaminants. Centrifuge the sample to separate the DNA from the cell fragments. RNase is used to destroy RNA. Alcohol is used to precipitate the DNA out of solution. Extracting DNA from plants is more challenging due to the pectin and cellulose fibers in the cell wall. To improve DNA extraction yield, freezing the plant tissue apparently ruptures the cell wall and a chloroform/isoamyl alcohol mix is used to remove the wall and other impurities from the plant DNA.

Adapted from: Biotechnology: Science for the New Millennium – Ellyn Daughterty
Inserting foreign DNA into plant cells: Small sections of foreign DNA must be added to one or more cell and then grown in culture. Unfortunately, plant cells do not take up naked plasmids like bacterial cells. Plant scientists have discovered a species of bacteria that lives in plants and is able to transfer plasmids from its own bacterial cells into the plant cells -- *Agrobacterium tumefaciens* contains a plasmid (Ti plasmid) responsible for the transformation process. Scientist learned how to grow *A. tumefaciens* and manipulate the Ti pDNA. The Ti plasmid can be cut open and genes of interest inserted. The recombinant Ti plasmid can be returned to *A. tumefaciens*, which will then transfer the recombinant plasmid into target plant cells.

Another technique to get pieces of DNA into plant cells is to use a “gene gun” – an apparatus that takes a plastic bullet covered with DNA-coated particles of gold and blasts them into plant tissue. As the bullet penetrates the tissue, some of the DNA-coated particles penetrate some cells which incorporate the DNA sections into the plant genome and start expressing the gene.

**Research:**
Use this site to answer the questions.
http://www.apsnet.org/publications/apsnetfeatures/Pages/Agrobacterium.aspx
1. Explain a little background about the *Agrobacterium tumefaciens*.
2. What are some unique characteristics of this bacterium?
3. What is the role of the Ti plasmid?
4. Briefly explain the genetic engineering of plants with this bacterium.

**Plant Proteins as Agricultural Products**
Many plants contain proteins of commercial value and recently scientists have learned how to engineer plants to make new proteins that confer desired qualities in crops. Scientists have even learned how to engineer plants to make human proteins for pharmaceutical use. (Remember: The phenotype of a plant is directly related to the proteins it produces – it is the DNA sequence that determines whether a certain protein will be made.)

- **Plant-based pharmaceuticals (PBP)s or Plant-made pharmaceuticals (PMP)s** – Most PMPs are proteins or other compounds that require certain regulatory proteins to be made. Advantages: Plants are easy to grow in large numbers and require less specialized equipment. Plants are eukaryotic and equipped to assemble complicated eukaryotic proteins. To produce a PMP – a plant is genetically engineered to make a protein normally not made in plants. The transformed plant is cloned and planted. When mature, plants are harvested and the recombinant human proteins are extracted and purified from plants.
- **Extracting protein molecules from plant cells** – Extracting DNA, RNA or proteins from cells is required for all biotech research, but extracting these molecules from plant cells presents special challenges. Challenges: Plants are dense and sometimes woody. Grating or mechanically breaking a plant sample increases the extraction yields. Grinding sample in liquid Nitrogen or on dry ice may also increase yields. Plant cells have thick, sticky cell walls that must be removed. Use cellulase and pectinase enzymes to break down the cellulose and pectin molecules in the cell wall. Freeze fracture techniques can be used to burst open plant cells.
- **Visualizing protein samples** – Gel electrophoresis can be used to identify the protein of interest. Cell extracts may be tested to reveal proteins with a particular activity.
Research:
Watch this video and answer the questions. https://www.youtube.com/watch?v=iiuAC0U9VxY
1. What are some plant-based pharmaceuticals being produced?
2. What are the advantages of using plants instead of bacteria?

Watch this video and answer the questions. https://www.youtube.com/watch?v=RBQZBWJgRT0
1. What disease is Professor Julian Ma research through the use of transgenic plants?
2. Briefly explain how the plant is transformed.
3. Once the plant is grown in the greenhouse, how do you see if the plant is producing the protein?
4. How do the scientists positively identify the protein?

Watch the video and answer the questions. https://www.youtube.com/watch?v=uCW6qeJt-JA
1. Briefly explain how the plants are being used to produce the Ebola vaccine.
2. What is biopharming? What are some benefits to biopharming?

Biotechnology in Food Production and Processing
Advances in agricultural biotechnology have impacted food production and processing resulting in improved food supplies, increased nutritional content of foods and increased food safety.

- Some of the biggest improvements in food yield and quality have come through advances in selective breeding of livestock and crops. Genetic testing and DNA fingerprinting allows selective breeding to increase the chance of producing offspring with desirable characteristics.
- Genetic testing and genetic modification are used to determine the best microorganisms in the fermentation process. Many foods are made with additives produced using genetic engineering.
- Protecting food crops and food products: Genetic modification to protect against drought, insects, and excessive temperatures. Food safety includes monitoring food during production and processing to test for allergens, and contamination. Cell cultures, DNA testing, protein testing and pathogen detection is standard in all food products.

The use of GM food products raises some concerns. The National Academy of Science takes the position that the potential risks associated with GMOs are the same as those created by traditionally bred organism. In the US, GMO and non-GMO crops and products are regulated by:

- Animal and Plant Health Inspection Service (APHIS) – division of USDA responsible for protecting agricultural products from disease/pests and responsible for field-testing GM crops.
- Food Safety and Inspection Service (FSIS) – division of USDA ensures all meat and poultry products meet high standards.
- Food and Drug Administration (FDA) – regulates the labeling of GM food. In the US, special labeling for GM food is not required unless a significant potential for food allergy or substantial change in nutrient composition or product identity is shown.
- Environmental Protection Agency (EPA) – regulates the use of pesticides/herbicides and determines safe use. Regulates biotechnology products that produce pesticides or herbicides.
- Food and Agriculture Organization of the United Nations (FAO) – supports an ongoing evaluation system that objectively determines the benefits and risk of each individual GMO.

Research:
https://www.bio.org/articles/plant-made-pharmaceuticals-background-and-key-points
1. How does the USDA and the FDA regulate PMPs?
2. Explain how developing therapeutic proteins in plants can be a safer, more efficient and cost-effective method of protein production.
MEDICAL Biotechnologies

Medical biotechnology includes all areas of research, development and manufacturing of medicine. Molecular biology techniques are used to develop diagnostic tools and therapies to improve patients' health and quality of life.

Drug Development

Drugs are chemicals that alter the effects of proteins or other molecules associated with a disease-causing agent and are usually specific for a particular disease process. Drug discovery is one of the fastest areas of medical biotechnology. Drugs may be harvested from nature, synthesized in a laboratory from organic molecules or genetically engineered. In all the drugs discovery strategies, numerous molecules are isolated and tested – this is screening. To isolate or design a drug that will treat a disease, scientists must understand the characteristics of the disease, how an organism contracts the disease and the course the disease takes in its host. A disease's origin and development, including its evolution, hosts, and means of transmission is called pathogenesis.

Sources of potential drugs: The study of drug composition, actions and effects is pharmacology. Understanding the pharmacology of a compound is one part of the drug discovery process – the goals is to find a compound that shows activity against one or more molecules associated with a disease. This process is lengthy and often tedious since millions of compounds may have to be screened as potential candidates. If a compound shows promise, it may have to be modified and tested repeatedly to meet FDA requirements.

Several medical compounds have come directly from plants or animals with very little modification. If the molecule cannot be found in nature, scientists have the ability to modify naturally occurring compounds to improve them. Simple organic molecules can be combined to synthesize larger organic molecules in a process known as combinatorial chemistry. Example: aspirin (acetylsalicylic acid) is the most frequently used pharmaceutical drug. Native people chewed willow bark and leaves for pain relief. The precursor to aspirin was found in the white willow tree and the active ingredient is salicin. Chemists converted salicin to salicylic acid which is less caustic than salicin (damaged soft tissues in mouth and stomach). Salicyclic acid was improved by addition of an acetyl group which produced an even gentler substance. Now scientists create a synthetic salicylic acid instead of harvesting willow trees.

Creating Pharmaceuticals through Combinatorial Chemistry

Sometimes researchers find natural compounds that have potential as drugs, but they must be modified in some way to be more effective or useful. Until recently, it has taken a chemist several months to isolate, create and test a single potential drug compound. Chemists have begun to use combinatorial chemistry along with parallel synthesis – making and testing many batches of similar compounds simultaneously to mass-produce and test thousands of potential drug candidates at the same time. High-throughput technologies aid scientists in developing and evaluating (screening) new compounds more quickly and efficiently.

Technicians keep track of compounds along with their test results in a “library”. A compound library may include hundreds of plates that are bar-coded so samples can be tracked and managed.

Microarrays can store samples -- a small glass slide or silicon chip with thousands of samples on it that can be used to assess the presence of a DNA or RNA sequence related to the expression of certain proteins. Recently, scientists have developed a technology called biochip (type of microarray) that can hold thousands of samples on a chip the size of a postage stamp.
Screening compounds – Screening and testing can take place in cells or with the use of lab instruments to analyze the molecular structure, activity, purity and/or concentration of the compound.

Research:
Explore the website: http://www.phrma.org/ Download the industry profile and read Chapter 4 & 5.

Choose any drug on the market and study it pathogenesis. Use the example of aspirin to help guide you. What is the drug’s origin and development?

Creating Pharmaceuticals through Peptide and DNA Synthesis
Proteins are long chains of amino acids that are folded into a functional unit. Peptide bonds are found between each amino acid. Peptides are amino acid chains – up to a few dozen amino acids in length which are too short to be folded into proteins. Peptides uses in medical R&D:

- Peptides can be used in an attempt to identify regulatory molecules. Regulatory molecules may control the manifestation of a disease. A peptide can be synthesized that will bind to a regulatory molecule and directly interfere with disease expression. If scientists understand the molecule they are trying to block, they can design and synthesize a peptide to try to block it.
- Peptides can be used as a vaccine antigen to initiate an antibody response.
- Peptides are often synthesized for use in the purification of other proteins. In affinity chromatography, peptides may be attached to chromatographic resin beads and used to bind and separate other proteins as a mixture flows through a column.

The development of peptide synthesizers automated the synthesis of peptides. Synthesizers can be programmed to run a column, adding the correct amino acids in the correct sequence to make a desired protein with a maximum length of 50 amino acids.

Oligonucleotides are small pieces of DNA – usually 50 nucleotides or less in length. Oligos may be used as primers, probes and recognition or blocker molecules. Oligonucleotides are critical in research for the identification of genes involved in disease or for use in disease prevention. Primers are produced by oligonucleotide synthesis for PCR and DNA sequencing applications. Primers are segments of about 25 nucleotides that can specifically bind to a single-strand DNA molecule. Primers are used to start synthesis and sequencing reactions. DNA synthesis is done on a DNA synthesizer – technology used to synthesize oligos similar to peptide synthesis. Creating antisense strands of nucleotides (strands that match noncoding regions of DNA – introns) gives researchers a way to block or interfere with gene expression.

Research:
Read the article: http://www.polypeptide.com/web/upload/medias/1401702726538c49464a6f5.pdf
1. What is the impact of peptide pharmaceuticals?
2. What are the current challenges associated with peptide pharmaceuticals?
3. What can we expect from peptide pharmaceuticals in the future?

Read the article: http://www.pharmamanufacturing.com/articles/2016/oligonucleotides-opportunities-pipeline-and-challenges/
1. What are the challenges associated with oligonucleotide pharmaceuticals?
2. What oligonucleotide drugs are in the pipeline?
Creating Pharmaceuticals by Protein/Antibody Engineering

Engineering cells to make antibodies is an expanding focus in medical biotechnology as scientists look for ways to improve a patient’s immune system to prevent or fight disease. Antibodies are large proteins with four large peptide chains bound together in the shape of a “Y”. The tips of the “Y” vary and are called variable regions. The tips of the antibody recognize and bind to other molecules called antigens. Antibodies are very specific, recognizing one or only a few different types of antigens. Antibodies are used to recognize molecules in medical research applications:

- Fluorescent dye molecules can be attached to antibodies and then the tagged antibody can recognize surface proteins on cells. In flow cytometry, fluorescent antibodies attach to surface proteins on certain cells. The flow cytometer can recognize and sort cells into separate vessels.
- Antibodies can be used in pharmaceutical manufacturing in the final step of purifying a protein product form cell culture. Antibodies are bound to the resin beads used in affinity chromatography columns and when a mixture of proteins is passed through the column, the antibodies bind to the protein pulling them out of the solution.
- Antibody-antigen reactions are important in disease prevention and companies produce antibodies or antigens for use in vaccines or vaccine research. A vaccine is something with an antigenic region that increases the production of antibodies against the compound. When antibody production increases in the body, the B cells make antibodies that remain in the body for a long time – called memory cells. Some memory cells stay in your body for all your life, or several years, but decrease in numbers. The vaccine provides long-term immunity from invading particles, because memory cells produce specific antibodies to eliminate the intruder. Antibodies for medical research and treatment are usually produced through genetic engineering of mammalian cells.

Medical biotechnologists have looked to livestock as production vessels for human pharmaceuticals. Animals that are genetically modified to make human pharmaceutical proteins are technically transgenic animals – called “pharm” animals. Benefits include – produce larger quantities, and at a lower cost. Transgenic animals are also being used to study medical disorders.

Research:
https://www.youtube.com/watch?v=fNhhAVaMZXE
1. What are therapeutic antibodies?
2. How does the therapeutic antibody work?
3. What are some future applications for therapeutic antibodies?

Recent Advances in Medical Biotechnology

- Several pathogens are not well recognized by antibodies. One of the newest therapeutic technologies is the development of antibody recruiting molecules (ARM). These molecules have a great affinity for a specific antigen and the antibodies that recognize them. The ARM assists the antibody binding by creating a bridge between the antigen and the antibody.
- Since no two people have the same DNA sequence, the response to different medications may differ between people. With knowledge of the human genome, scientists will be able to design and modify drugs to better meet an individual’s needs – prepare a pharmaceutical regimen personalized for each patient. Pharmacogenetics involves utilizing genetic and protein codes to design or improve medications. It applies advances in PCR, sequencing, microarrays, proteomics and other technologies to create new, personalized therapies – personalized medicine. Allows for individual drug design that could reduce the risk for allergic reactions and side effects and increase drug effectiveness.

Adapted from: Biotechnology: Science for the New Millennium – Ellyn Daughterty
One tool needed for personalized medicines and genetic diagnosis is the ability to map individual patient’s genes and protein expression so scientists can look at the differences between people who are sick and people who are not sick. The differences are markers – **biomarkers**. Biomarkers may be sections of DNA or proteins in a certain cell or a compound that may be used to measure some aspect of health or treatment. Biomarkers may be used to diagnosis or treat disease and monitor the effectiveness of a treatment. Some examples of markers – proteins that is monitored in blood or urine; gene sequence present only in sick patients; protein on surface of cancer cells; molecule found in higher concentrations after medical treatment, etc....

**Monoclonal antibodies** are produced in special cells called hybridomas. Produced by fusing immortal tumor cells with specific antibody-producing WBCs (B cells), hybridomas grow rapidly, making large amounts of a specific antibodies that were coded for in the original B cells. The advantage is that many identical antibodies to specific antigens are produced in large quantities.

**Gene therapy** has the potential to treat and cure diseases that are inherited or genetic in origin. Functional genes are used to replace or improve the function of a defective gene or new genes with new functions are used to correct medical conditions.

Artificial tissue or organs could replace or augment the function of damaged ones.

Biomedical instrumentation to transmit images and data to physicians, etc....

Regenerative medicine describes efforts to restore the function of diseased or damaged tissue.

**Research:**
Research two of the recent advances in medical biotechnology. Write a brief summary about the advancements.

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**Biotechnology Research and Applications**

**Genomics** is the study of an organism’s genome -- includes the nucleotide sequence, how the sequence is read and regulated, and how the sequence and genes vary. Once the genome is sequenced, it genes, gene functions and gene regulators are studied and compared. The goal is to understand the relationship between genomes and protein expression.

Scientist use automated sequencers and bioinformatics to analyze the relationship between genes and protein expression. The ability to sequence large amounts of DNA requires the **shotgun cloning method** – allows all DNA from an organism to be prepared and sequenced fairly rapidly. Restriction enzymes are used to splice DNA released from cells into smaller pieces. The pieces are ligated into plasmids to create a library of genome fragments. The plasmids are used to transform cells and make many copies of the recombinant DNA (rDNA) plasmids. Sequencing and computer analysis determines how the pieces fit together.

The **Human Genome Project** resulted in identifying gene sequences and protein targets for pharmaceuticals, gene therapies and genetic testing. Genome research has led to increased understanding of evolutionary relationships between organisms.

**Microarray technology** was developed in parallel with the deciphering of the human genome. A microarray is a collection of oligonucleotide sequence that are linked at fixed locations on some type of glass slide, silicon chip or nylon membrane. The array sequences are used as “probes” to recognize other nucleotide sequences of interest. The sequences are labeled with fluorescent tags for visualization. Microarrays may be used to for both genetic screens and testing for disease and gene expression studies.
**Bioinformatics** is the use of computers and statistical analysis to understand biological data. Large amounts of numerical and sequence data are collected, the data may be organized into biological databases.

**PCR** makes it possible to isolate small sections of genetic information in a DNA sample. PCR is used for gene identification, disease detection, genetic fingerprinting and gene synthesis. Traditional PCR was limited to replication of DNA. Two new PCR applications: (1) Reverse-transcription PCR: The enzyme found in retroviruses may be used to produce a copy of DNA (cDNA) form a single-strand RNA. The cDNA is the exact genetic code for the mRNA transcript and can be amplified using PCR. RT-PCR is used to detect and measure mRNA in sample as a way to understand gene expression and the proteins. (2) Real-time PCR process uses fluorescently tagged probe technology to visualize and quantify the PCR product as it is made.

Several forms of **RNA** interact with DNA and other RNA molecules. This interaction turns genes on and off, and interferes with processing and translation of RNA at ribosomes. Useful forms of RNA can be constructed in the lab for the very purpose of gene recognition and gene blocking. Several types of regulatory RNA include RNAi, siRNA, and microRNA. RNAi (RNA interference) are double-stranded RNA molecules that enter cells and mimic viral DNA invasions. The cells respond by chopping the RNA into small pieces. The short, double-stranded RNA pieces unzip, and single strands then bind with proteins that interfere with the cells native RNA or DNA – blocking protein production. siRNA (short-interfering RNA) is essentially the same as RNAi, except scientists actually create the short-interfering single RNA strands to learn which genes are turned off and which proteins are not synthesized due to siRNA activity. The siRNA may also bind with native RNA, and cells may mistakenly destroy the complex. MicroRNA molecules are small pieces of RNA that are known to intercept posttranscriptional RNA function. MicroRNA molecules bind to mRNA and immediately prevent the mRNA from being translated.

**Northern blots** are used to study the presence and concentration of certain types of RNA in cells. The RNA extracts are run on a gel, transferred to a membrane and then visualized. Researchers can learn how and where RNA is produced and active. RNA researchers are using microarrays to study the entire transcriptome (all the mRNA in a cell). Transcriptomes indicates what genes are being expressed and what proteins are being made.

**Proteomics** is the study of protein activity. It includes the study of RNA splicing and other posttranscriptional modifications. Methods for studying include mass spectrometry, nuclear magnetic resonance (NMR), protein arrays, protein assays and Western blots.

**Research:**
Conduct research on the following topics: regenerative medicine, bioremediation, and biofuels. Write a brief summary about each topic.

**HS-AB-3: Demonstrate how advanced techniques in biotechnology contribute to our quality of life.**
3.1 Describe how biotechnology has contributed to the advancement of biology impacting human well-being, such as disease management through vaccines, food production, materials science and molecular identification.
3.2 Apply biotechnological techniques to forensics including materials analysis, DNA fingerprinting and sample collection.
3.3 Utilize biotechnology for healthcare applications.
3.4 Utilize biotechnology for diagnostic applications (e.g. hepatitis, HIV, BRAC, rapid streptococcus).
3.5 Explain the role of biotechnology in therapeutics (e.g., gene therapy, vaccines, antibody therapy, cell therapy).
3.6 Describe how bioinformatics can be used to predict disease and determine treatment.
3.7 Investigate the principles of genetic mapping applied to healthcare or phylogenetics and evolution
3.8 Describe the non-medical applications of biotechnology, including enzyme production, biofuel and biomaterials discovery and manufacturing.

Adapted from: Biotechnology: Science for the New Millennium – Ellyn Daughterty