Biotechnology

Applying science knowledge and new technology to our 21st century needs

1950s

- Scientists had concluded that DNA is the genetic material
- But…
  - The field of Genetics was just beginning
  - The field of Biotechnology did not yet exist!!

Nucleotides > Genes > Chromosomes

- Researchers must be able to work with DNA without being able to see it or handle it directly
  - It's too small!
- A whole chromosome is still too large to study all at once, there's just too much information

ONE OF THE FIRST THINGS TO CONSIDER IN BIOTECHNOLOGY AND GENETICS RESEARCH IS HOW TO PRECISELY CUT DNA

Why would we want to cut DNA?

Human DNA is approximately 3 billion base pairs long, and we have about 30,000 different genes.

In order to study a specific gene we need to cut down the long strand of DNA and select only the specific gene or region that we are working on

Restriction Enzymes = "molecular scissors"

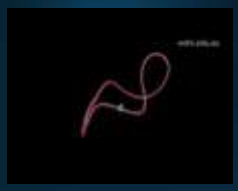
Image source: Microsoft Clipart Gallery

Bacteria are the factory workers of Biotech

- Bacteria can be infected by viruses, as a defense mechanism they produce Restriction Enzymes to cut up the foreign DNA of the virus.
- Restriction Enzymes only cut a strand of DNA at a specific sequence called a restriction site or recognition site.
- There are 100s of known restriction enzymes
- Restriction sites are 4-8 base pairs long.

Restriction Enzyme & DNA Ligase

- **Restriction enzyme (blue)**: cuts DNA---leaves "sticky ends"
- **DNA Ligase (green)**: "glues" DNA back together



Cuts can be 'blunt' or 'sticky'

Restriction Enzyme – Eco R1

Recognition Site

ACTGGTACGAATTCCGTA
TGACCATCCTTAAGGCAT

ACTGGTACG
TGACCATCCTTAA

AATTCCGTA
GGCAT

Sticky Ends!

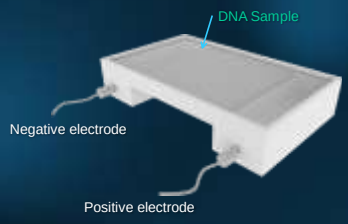
Sticky ends will bind to complementary bases that come along, making them useful

Once DNA has been cut into fragments by restriction enzymes, the fragments can be sorted or separated by size

HOW?

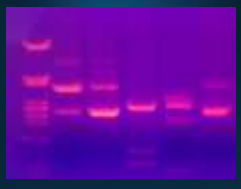
Gel Electrophoresis

- An electrical current is used to separate a mixture of different fragments of DNA



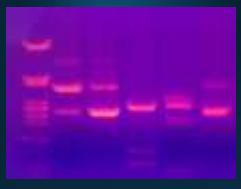
Gel Electrophoresis

- Because DNA has a negative charge, the fragments move toward the positive electrode
- Pores in the gel allow small molecules to move through the gel quickly, while large molecules get "stuck" and move slowly



Gel Electrophoresis

- So, the length of a DNA fragment can be estimated compared to other fragments by measuring how far each fragment migrated through the gel
- DNA fragments appear as different bands or lines on the gel (after staining)

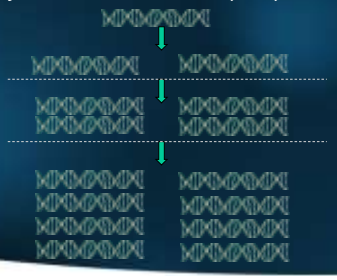


Restriction Maps

- The band pattern can be thought of as a map of the original strand of DNA
- The map shows the lengths of DNA fragments between restriction sites on a DNA strand

How can we get a large enough sample of DNA to work with?

- Polymerase Chain Reaction (PCR)

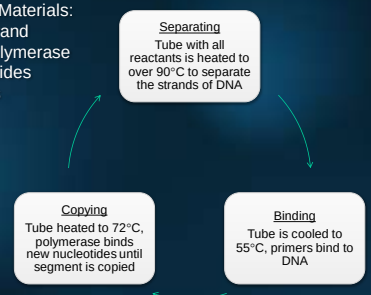


Polymerase Chain Reaction (PCR)

- A technique that produces millions (or billions!) of copies of a specific DNA sequence in hours
- Invented by Kary Mullis in 1983
  - He patented the process and sold the rights for $300 million
  - Won the Nobel Prize in Chemistry in 1993
- Under the right set of conditions, DNA polymerase enzymes will make new copies of DNA in a test tube, just like they do in your cells!

Polymerase Chain Reaction (PCR)

4 Starting Materials:
- DNA strand
- DNA polymerase
- Nucleotides
- Primers



Polymerase Chain Reaction (PCR)

- Each PCR cycle doubles the number of DNA copies
- 1 > 2 > 4 > 8 > 16 > 32 > 64 > 128 > 256 > 512…
- After 30 cycles, more than 1 billion copies!

- What is a primer? A short segment of DNA that acts as a starting point for the new strand

DNA Fingerprinting – a restriction map!

- A representation of parts of an individual's DNA that can be used to identify a person at the molecular level
- A DNA sample is cut with restriction enzymes and a restriction map is created on a a gel
  - When several DNA samples are treated with the same restriction enzymes, their fragments can be compared side-by-side on the same gel
  - Since our DNA sequence can't change, the restriction map for 1 person will always be the same when treated with the same enzymes

DNA Fingerprinting – a restriction map!

Our DNA is 99.9% identical, how do we find the differences?

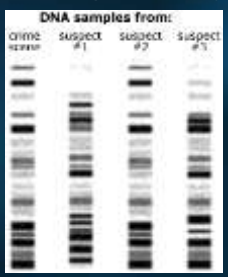
- The greatest differences in DNA sequence between people are in the non-coding sections of our DNA (introns)
- So….DNA fingerprinting focused on the introns, which often include stretches of nucleotide sequences that repeat several times
- Each person's DNA differs in how many repeats they have

DNA Fingerprinting – a restriction map!

- DNA fingerprinting has become reliable and widely used in US courts since the 1990s
- In most forensic cases, at least 5 regions of DNA are analyzed
- This technique can be used to:
  - Prove guilt or innocence
  - Prove family relationships
  - Identify victims

DNA Fingerprinting

- Side by side comparison of genetic profiles



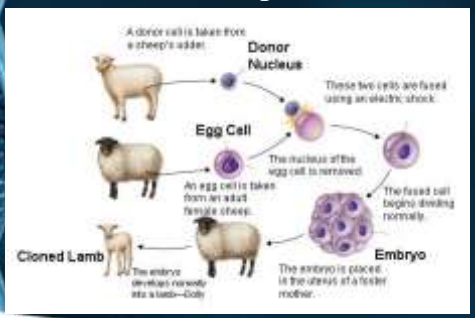
Genetic Engineering – changing DNA!

- Clone – a genetically identical copy of a gene or organism
- Uses:
  - Organs from cloned mammals
  - Endangered species
- Gene cloning – making a copy of that segment of DNA that codes for something (a gene)

Hello Dolly

- Created through **CLONING**

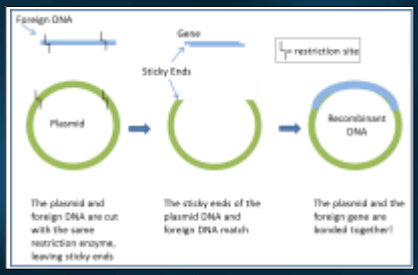
How Dolly?



Recombinant DNA

- Contains genes from more than 1 organism
- Plasmids – tiny rings of DNA, found in bacteria, that separate from the bacterial chromosome and replicate on their own
- Bacteria will replicate foreign DNA along with their own!

Recombinant DNA



Bacterial Transformation

- Bacteria are turned into gene factories when plasmids containing specific genes are put into the bacteria
- The bacteria will:
  - Replicate the plasmid (make lots of copies)
  - Express the gene or trait that is on the plasmid!
- We call these bacteria "transformed" cells

Transgenic Organism

- Contain one or more genes from another organism
- In plants, transgenic bacteria infect the plant, the new gene becomes part of the plant's DNA
- Example:
  - Resistance to frost
  - Resistance to disease
  - Resistance to insects

Transgenic Organisms

Glow in the dark tobacco plant (luciferase gene from firefly)

Antifreeze protein from fish in strawberries

Genetically Modified Organisms

An organism whose DNA has been altered using genetic engineering technology

Benefits to GMO Food

Increased shelf life
Increased resistance to herbicides
Increased nutritional value
Ability to grow or live in harsh conditions
Grow more with less land

FEED THE WORLD!

Benefits to GMO Animals

Higher resistance to disease
Produce "better" milk, eggs, and meat
Less maintenance or upkeep costs

Controversies Against GMOs

Allergic reactions to proteins produced

Antibiotic ingestion

Escape into the natural world and breed with native varieties

Less diversity

Genomics

- The study of genomes, which can include the sequencing of all of an organism's DNA
- Scientists can compare genomes both within and across species to find similarities and differences
  - Find genes that cause disease
  - Evolutionary relationships
- Gene sequencing – determining the order of DNA nucleotides (DNA sequence) of genes or genomes

Human Genome Project – 1990s

Goals:

1. Map and sequence all of the DNA base pairs of the human chromosomes
   a) 3 billion base pairs!
   b) Finished in 2003
2. Identify all of the genes within the sequence

Genetic Screening and Gene Therapy

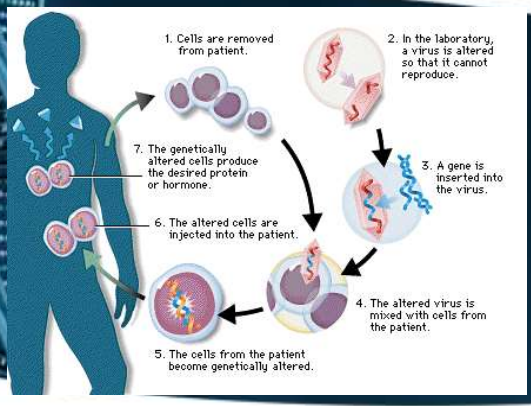
- Genetic screening – testing DNA to determine a person's risk of having or passing on a genetic disorder
- Gene Therapy – replacement of a defective or missing gene, or addition of a new gene, into a person's genome

Mostly Experimental!

Gene Therapy

- The introduction of genes into cells to compensate for abnormal genes or to make a beneficial protein.
- If a mutated gene causes a necessary protein to be faulty or missing, gene therapy may be able to introduce a normal copy of the gene to restore the function of the protein.

Gene Therapy



Gene Therapy Examples

- Severe Combined Immunodeficiency (SCID)
  - Absence of functional immune system
  - "Bubble boy disease"

Gene Therapy Examples

- Cystic Fibrosis
- Defective CFTR gene
- Deletion of three nucleotides