Restriction Enzymes Can Cut DNA into Precise Fragments

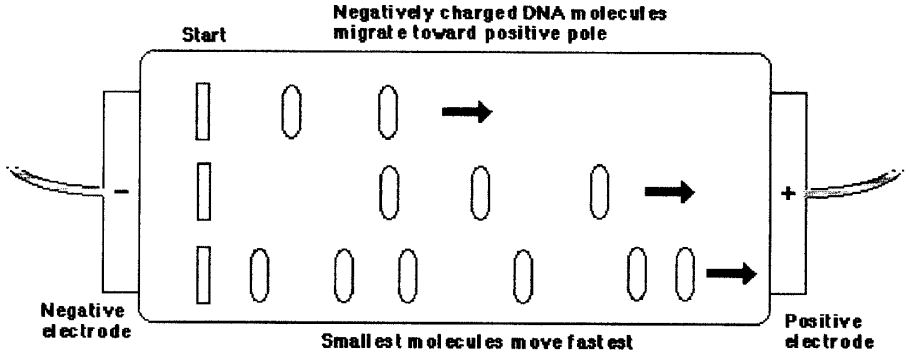
- Restriction enzymes cut only at specific sequences of 4 to 8 bases
- A long restriction site is rare. Cutting rare sites will produce a few large pieces of DNA.
  - Restriction enzymes with long sites -> small number of large fragments
  - Restriction enzymes with short sites -> large number of small fragments

Number of bases	Probability	Average Number of Fragments for 1 Million Bases	Average Size of Fragment (Base Pairs)
4	1/256	3907	256
5	1/1024	978	1024
6	1/4096	245	4096
7	1/16384	62	16384
8	1/65536	16	65536

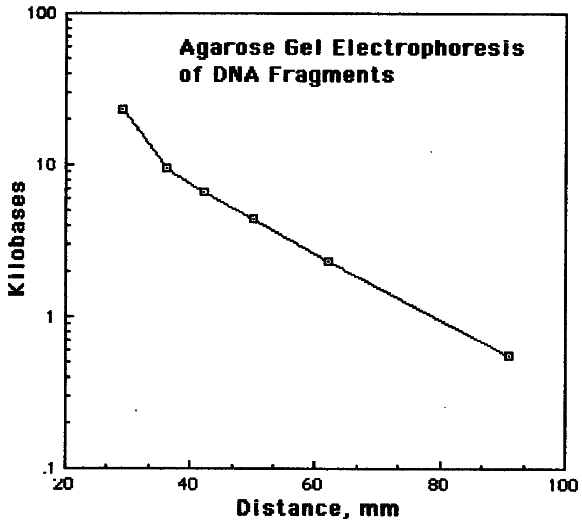
- There are now about 200 known restriction enzymes
- Some uses for restriction enzymes:
  - Sequencing DNA: restriction enzymes cut different copies of DNA at exactly the same spot
    - Large amounts of the same fragment can be obtained for analysis
  - Cloning DNA: restriction enzymes allow formation of recombinant DNA

Fragment Size Can be Analyzed by Gel Electrophoresis

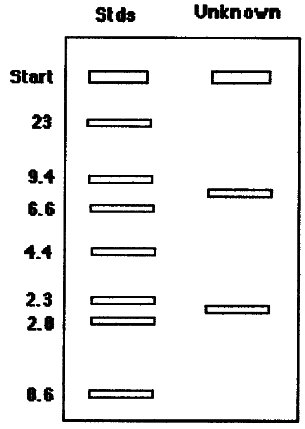
- DNA has a negative charge, and if placed in an electric field will migrate toward the positive pole



- Small fragments will move faster than large ones, according to a logarithmic relationship, in agarose gels



- This can be used to determine the sizes of DNA fragments in experiments
- Methods to make the DNA visible:
  - Autoradiography: radioactive DNA; gel exposed to a photographic plate to make bands visible
  - Stain with ethidium bromide: fluorescent dye- must be handled carefully because it is carcinogenic
  - Stain with methylene blue- not as sensitive as ethidium, but safer
- Example:

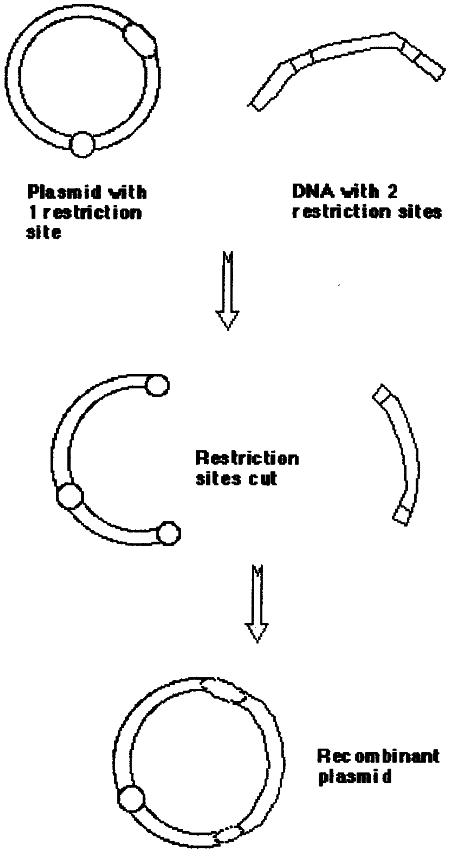


- The example shows calibration standards, running from 0.6 to 23 kilobases, on the left
- The unknown has 2 bands, of approximately 7.5 and 2.1 kilobases

Restriction Enzymes Can be Used to Make Recombinant DNA

- If a plasmid and the DNA sample of interest have some of the same restriction sites the DNA can be inserted into the plasmid
- Steps in making recombinant DNA:
  - Cut both the plasmid and the DNA sample with the same restriction enzyme

        - This will produce "sticky ends" on both plasmid and DNA sample
    - The sticky ends are complementary and will hydrogen bond to each other
    - Make the attachment permanent with the enzyme DNA ligase (catalyzes formation of phosphodiester bonds

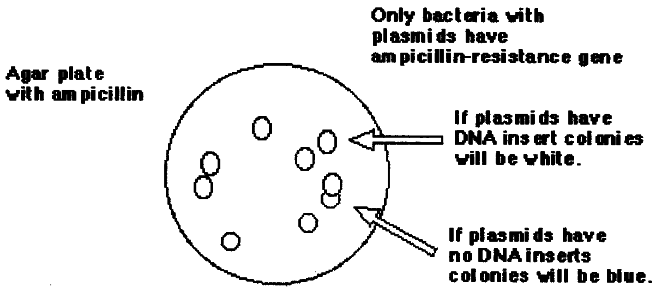


- The blue dot on the plasmids represents the ampicillin-resistance gene
- Similar methods can be used to make recombinant DNA with bacteriophage
- Recombinant DNA was first made by these methods by Herbert Boyer (Stanford University) and Stanley Cohen (UC, San Francisco) in 1973

Several Tricks are Used to Detect Recombinants

- The recombinant plasmids are put into bacteria so that multiple copies can be made
- When the bacteria divide the plasmids divide too
- If bacteria are diluted and grown on agar plates they will form round colonies
- Each colony is a clone derived from a single cell
- Tricks are used to find colonies with DNA inserts
    - Trick 1: plasmids with a gene that gives resistance to the antibiotic ampicillin are used
        - If the bacteria are grown on agar plates containing ampicillin only bacteria with a plasmid can grow
    - Trick 2: DNA is inserted at a restriction site in the middle of a beta-galactosidase gene (Lac Z)

- If DNA is inserted the gene is destroyed
- Bacteria with beta-galactosidase gene can convert an artificial substrate, X-gal, into a blue dye
- If the colonies are given X-gal those without recombinant DNA will be blue (their gene works) and those with recombinant DNA will be white (their gene has been destroyed)



The Nuclear DNA Can be Cut up to Make a Genomic DNA Library

- If all of the DNA in the nucleus is cut up with restriction enzymes and made into plasmid recombinant DNA thousands of different fragments will be obtained
- This is called a genomic DNA library since it contains all of the DNA from the genome
- Bacteria will not be able to make proteins from a genomic library because they do not know how to cut out introns
- If proteins are required the DNA can be grown in eukaryotic cells

Messenger RNA Can be Used to Make a Complementary DNA Library

- Another way of making a DNA library is to start with messenger RNA
- The m-RNA can be converted to double strand DNA using the enzyme, reverse transcriptase
- The DNA can then be put into plasmids to make a complementary DNA library
- This type of library shows the genes that are actually being transcribed into m-RNA
- Bacteria can make proteins from a cDNA library because there are no introns

PCR – Polymerase Chain Reaction: This process is used to make multiple copies of DNA when only a small amount is available. Scientists can then run many tests with the small amount of DNA they have found. Examples: wooly mammoth DNA analysis, prenatal testing, human genome project, and forensics.

RFLP analysis:
1. DNA segments with different alleles of a gene result in dissimilar banding patterns
2. the restriction fragment length differences are termed restriction fragment length polymorphisms (RFLP's)
3. readily detected with recombinant DNA methods and can be used as genetic markers
4. usefulness:
    a. location of disease genes (linkage to known RFLP's)
    b. genetic fingerprint of individuals used in forensics

Importance of DNA Technology:
1. medicine – medication will be able to eventually be genetically suited to individuals.
2. diagnosis of disease – locating where diseases are on genomes.
3. human gene therapy – one day being able to correct inherited genetic disorders.
4. forensics – determining criminals from DNA evidence.
5. environmental impacts – creating creatures that can transform pollution.
6. agriculture – a) animal husbandry (creation of transgenic organisms)
    b) plants – creating insect resistant or pesticide resistance plants

Cloning

Cloning has occurred since the first prokaryotic cell. It is the creation of an exact genetic copy of an organism. Bacteria reproduce this way.
Some plants can be split and replanted as two separate plants. They are also clones.

Cloning mammals is the new frontier in cloning.

The Process of Cloning Dolly

1. Mammary gland cells were taken and grown with little nutrients to cause them to revert to the G0 phase. They dedifferentiated.
2. Egg cell take from a sheep and nucleus removed.
3. The dedifferentiated cell was injected into the egg. The combined cell was then electric shocked to get it to start to divide.
4. Embryo implanted into a third sheep's uterus after 6 days.
5. Embryo developed into a lamb identical to sheep that donated the mammary gland cell.

***NOTE- Dolly is not an exact copy because the mitochondrial DNA came from the egg donor.

Differentiated cells have already had their DNA turned "on" or "off" to program them for what type of cell they will become.

Cloning opens up an entire ethical controversy.
What are the two viewpoints for it?