

## TOPIC - New Frontiers in Genetics

### I. Basic Strategies

#### A. Recombinant DNA and Gene Cloning

##### 1. Restriction enzymes

- a. occur naturally in bacteria (protection from the DNA of other organisms)
- b. restriction: foreign DNA is cut into small segments
- c. recognition sequences: short, specific nucleotide sequences recognized by restriction enzymes
- d. bonds of DNA are cut in a staggered manner called sticky ends
- e. different DNA pieces are then joined by DNA ligase
- f. gel electrophoresis: separates the restriction fragments after DNA has been treated with restriction enzymes
  1. separated by size and electric charge
  2. DNA molecules can be identified by specific band patterns
  3. also, isolation and purification of interesting genes (still maintain biological activity)

##### 2. Gene-Cloning Steps:

- a. isolation of two kinds of DNA (i.e., bacterial and foreign DNA)
- b. treatment of the plasmid and foreign DNA with the same restriction enzyme (cuts both at the same restriction site)
- c. mixture of foreign DNA with clipped plasmids
  1. sticky ends of the plasmid base pair with complementary sticky ends of foreign DNA fragments
- d. addition of DNA ligase
  1. catalyzes the formation of covalent bonds forming recombinant DNA
- e. introduction of recombinant plasmid into bacterial cells
  1. taken up by bacteria via transformation
- f. production of multiple gene copies by gene cloning
  1. bacteria reproduce, cloning the inserted gene in the process

#### B. DNA Sequencing

##### 1. synthesis of artificial genes in the lab (insulin)

###### The Sanger Method

restriction enzymes are used to cut the DNA into restriction fragments....

- a. a preparation containing one of the strands of the DNA fragment is divided into 4 portions
  1. each portion contains: a radioactive primer, DNA polymerase, and the 4 DNA triphosphates
  2. each mixture also contains one of the 4 nucleotides in a modified (dideoxy - dd) form
- b. new strand synthesis begins with the radioactive primer and continues until a dideoxyribonucleotide is incorporated
  1. a set of radioactive strands is produced
- c. the new DNA strands are separated by gel electrophoresis
  1. the radioactive bands can be read and the original template is deduced

#### C. Polymerase Chain Reaction (PCR)

1. allows any piece of DNA (even minute quantities) to be quickly amplified (copied many times) in vitro
  - a. DNA is incubated with special primers and DNA polymerase molecules
  - b. billions of copies are produced in a few hours
    1. useful for the following:

- a. analysis of ancient DNA (woolly mammoth)
- b. forensics
- c. prenatal diagnosis
- d. viral genetic detection (HIV)
- e. human genome project

#### D. 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

#### E. Importance of DNA Technology

1. Diagnosis of Disease
2. Human Gene Therapy
3. Vaccines
4. Pharmaceutical Products
5. Forensics
6. Agricultural Uses

## II. Mutations revisited.....

*Mutation:* an inheritable change in the DNA sequence of a chromosome

### A. Gene Mutations

*Point mutation* (base-substitution)- a change in the DNA sequence involving only a single nucleotide substitution (*sickle-cell anemia*)

*Missense mutation:* point mutation with varying effects-

- a) may code for protein alteration or destruction;
- b) may result in silent mutations (undetectable)

*Nonsense mutation:* point mutations that change an amino acid-specifying codon into a termination codon

*Frame shift mutation-* a change in the DNA sequence involving the addition or substitution within the gene (usually a new sequence of amino acids are coded)

### B. Chromosomal mutations

(*aneuploides*- extra or absence of chromosomes)

*translocations-* one chromosome may break off and attach to nonhomologous chromosomes, or two nonhomologous chromosomes may exchange parts

- a) genes missing - *deletions*
- b) extra copies of genes - *duplications*

*nondisjunction-* failure of chromosomes to separate at anaphase (in meiosis, either anaphase I or anaphase II); creates a mixture of normal and abnormal cells

## C. Detection

*amniocentesis*: sampling of the amniotic fluid surrounding a fetus to obtain information about its development and genetic make-up

*chorionic villus biopsy*: sampling of the extra-embryonic cells that are genetically identical to the cells of the embryo (genetic assessment)

*karyotype*: the chromosomal constitution of an individual

## D. Disorders

*Down*: Trisomy 21; 1/700 births; usually 40+ women

*Turner*: XO genotype

*Klinefelter*: XXY genotype

## Quick Notes:

Recombinant DNA (genetic engineering): portions of DNA from different sources are modified and inserted into other cells where the genes carried by the modified DNA are expressed

- Scientists need segments of DNA small enough to manipulate, large quantities of the segments, know the nucleotide sequence in the segment, able to identify the specific segments in which they are interested
- uses plasmids, viruses, and transposons to move genes

Restriction enzymes: enzymes that cut foreign DNA into small pieces before they can be replicated

- Different enzymes cut DNA at different sites

Recognition sequences: specific nucleotide sequences which restriction enzymes cut through

DNA ligase: enzyme that catalyzes the reaction that links sticky ends together

Clones: genetically identical bacteria and other organisms produced asexually from a single parent cell or organism

- Polymerase chain reaction (PCR): process for producing multiple copies of DNA segments
- DNA sample added to solution of DNA polymerase molecules and DNA primer molecules  
Solution is heated and DNA helix separate ( Solution is cooled and the primers attach to their complementary sequences ( DNA polymerase molecules recognize the primers and add nucleotides at the places where primers are attached ( Both strands of DNA are copied)
- Process done many times to get more segment copies
- Copies analyzed to determine nucleotide sequence of each fragment

Nucleic acid hybridization: "magnet" used to locate and isolate DNA

Somatostatin: first human protein synthesized in a bacterial cell

- Had same activity as natural hormone
- Paved the way for bacterial synthesis of medical proteins (insulin, clotting factors for hemophilia, human growth hormone, vaccines against viral diseases)

Sickle-cell anemia

- Different hemoglobin than normal people
- Longer DNA fragment in sickle-cell DNA than in normal

Restriction-fragment-length polymorphisms (RFLPs): genetic markers