DNA Technology – Ch20

-	Genetic engineering:	manipulation of genes for	r purposes
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- <u>Recombinant DNA</u>: DNA in which genes from ______ are linked
- <u>Biotechnology:</u> manipulation of ______ or their components to perform practical tasks or provide useful products

DNA Cloning

- Restriction enzymes (endonucleases): in nature, these enzymes ______ bacteria from intruding DNA; they cut up the DNA (restriction); very ______
- Restriction site: ______ for a particular restriction enzyme
- Restriction fragments: ______ of DNA cut by restriction enzymes in a reproducible way
- Sticky end: ______ of restriction fragments
- DNA ligase: enzyme that can ______ the sticky ends of DNA fragments
- Cloning vector: DNA molecule that can carry ______ into a cell and replicate there (usually bacterial ______)

Steps of Eukaryotic Gene Cloning

- Isolation of cloning vector (bacterial plasmid) & gene-source DNA (______)
- Insertion of gene-source DNA into the cloning vector using the same restriction enzyme;
 the fragmented DNA with DNA ligase
- Introduction of cloning vector into ______ (transformation by bacterial cells)
- _____ of cells (and foreign genes)
- Identification of cell clones carrying the gene of interest

Storing Cloned Genes

- Genomic libraries: ______ of plasmid containing cell clones
 - Cloned genes can also be stored in phages
- cDNA: DNA made from mRNA that contains the _____ coding sequence of a gene (no introns)
- <u>Nucleic acid hybridization</u>: using a complementary DNA strand to attach to a gene of interest in a genomic library
 - _____ probe attaches and then a photographic film is used to view where the gene is located in the _____

Polymerase chain reaction (PCR)

_____ of any piece of DNA _____ cells (in vitro)

- Materials: heat, DNA polymerase, nucleotides, single-stranded DNA primers
- Applications: fossils, forensics, prenatal diagnosis, etc.

DNA Analysis <u>Gel electrophoresis</u>: separates nucleic acids or proteins on the basis of ______ or creating DNA bands of the same length

Southern Blotting

- Used when there are ______ pieces of DNA fragments to be seen with normal gel electrophoresis
- Combination of gel electrophoresis and nucleic acid hybridization
- Uses: identifying carriers of ______

DNA Sequencing and Gene Expression

- Determination of ______ sequences (Sanger method, sequencing machine)
- Genomics: the study of genomes based on DNA sequences
- Human Genome Project
- <u>Dideoxy Chain Termination Method</u>: using a single strand of DNA to determine the exact sequence of the DNA strand
- <u>RT-PCR</u>: taking ______ from developmental stages of organisms to determine which genes are active at different times
 - Pull out the mRNA, make DNA strand, ______ specific gene of interest, and use gel electrophoresis to see the strands

Restriction fragment analysis

- Restriction fragment length polymorphisms (RFLPs): ______ of restriction fragments that are used to distinguish between individuals
- DNA Fingerprinting
 - _____(STRs): variation from person to person; faster the RFLP analysis

Animal Cloning

- Steps
 - Mammary cell donor DNA is added to an _____ striped of its DNA
 - Cells are fused and grown in culture
 - o ______ is added to surrogate mother
 - Baby is genetically ______ to mammary cell donor

Practical DNA Technology Uses

- Diagnosis of disease
- Human gene therapy
- Pharmaceutical products (vaccines)
- Forensics
- Animal husbandry (transgenic organisms)
- Genetic engineering in plants
- Ethical concerns?