

Tools of the Trade Genetic Techniques and Methodologies

Tools and Techniques

- Enzymes
- Analysis of DNA
- Nucleic acid hybridization
- Synthesizing DNA
- Polymerase Chain Reaction

Enzymes

- Restriction endonuclease
- Ligase
- Reverse transcriptase
 - cDNA

Restriction endonuclease

- Originates in bacterial cells
- Many different types exist
- Natural function is to protect the bacterium from foreign DNA (bacteriophage)
- Recognizes 4 to 10 base pairs (palindromic sequence)
- Cleaves DNA at the phosphate-sugar bond
- Used in the cloning method
- Ex. *EcoRI* from *Escherichia coli*

Ligase

- Link DNA fragments
- Rejoin the phosphate-sugar bonds
- Used in the cloning method

Reverse transcriptase

- Converts -ssRNA to cDNA
- Complementary DNA (cDNA)
 - Required for eukaryote gene expression
 - mRNA to cDNA
 - No introns are present

Analysis of DNA

- Electrophoresis
- Hybridization and probes
- Sequencing
- Polymerase Chain Reaction

Electrophoresis

- Separation of DNA based on size
- Negative charge DNA (phosphate group) migrates to positive electrode
- Usefulness

- Characterizing DNA fragment
- Fingerprinting

Hybridization and probes

- Complementation
- Probes
- Southern Blot

Complementation

- Complementary sites on two different nucleic acids bind or hybridize
 - DNA hybridize with DNA
 - DNA hybridize with RNA
 - RNA hybridize with RNA

Probes

- Small stretches of nucleic acid with a known sequence is called an oligonucleotide
- Single stranded
- Detects specific nucleotide sequences in unknown nucleic acid samples

Southern blot

- Method for detecting an unknown sample of DNA
- Incorporates restriction endonuclease, electrophoresis, denaturing, probing, and visual detection.

DNA Sequencing

- Provide the identity and order of nucleotides (bases)
- Method
 - Sanger method
 - Dideoxy or capillary sequencing
 - Polymerase adds fluorescent nucleotides one-by-one
 - Sequences a single fragment at a time
 - Next Generation Sequencing (NGS)
 - Same idea but sequences millions of fragments at a time

Polymerase Chain Reaction (PCR)

- Specific amplification of DNA
- Involves a denaturing, priming (annealing), and extension cycle
- 30 cycles are sufficient for detection/amplification of DNA
- Can be used to detect disease or infectious agents

Recombinant DNA

- Recombinant DNA
- Applications
- Cloning vectors
- Cloning host
- When a cloning host receives a vector containing the gene of interest
- A single cloning host containing the gene of interest is called a clone

Applications

- Protein production
- Alter organism's normal function
- Source of DNA (synthesis)

Cloning vectors

- Carry a significant piece of the donor DNA (gene of interest)
- Readily accepted DNA by the cloning host
- Contain an origin of replication
- Contain a selective antibiotic resistant gene
- Ex. Plasmids, phages

Cloning host

- Bacteria (prokaryote)
 - *Escherichia coli*
 - Bacteria will not excise introns from eukaryotic DNA
- Yeast (eukaryote)
 - *Saccharomyces cerevisiae*
 - Will excise introns

Recombinant Organisms

- Modified bacteria and viruses
- Transgenic plants
- Transgenic animals

Modified bacteria

- *Pseudomonas syringae*
 - Prevents frost crystals from forming on plants
- *Pseudomonas fluorescens*
 - Contains an insecticide gene

Transgenic plants

- *Agrobacterium tumefaciens*
 - Ti plasmid contains gene of interest, and is integrated into plant chromosome
 - Ex. tobacco, garden pea, rice

Transgenic animals

- Knockout mouse
 - Tailor-made genetic defects
 - Cystic fibrosis
 - Gaucher's disease
 - Alzheimer's disease
 - Sickle-cell anemia
 - Pharmaceutical production

Therapy

- Gene therapy
- Antisense and Triplex DNA

Gene therapy

- Repair a genetic defect
 - *Ex vivo* strategy
 - *In vivo* strategy
- Severe immunodeficiency disease
- Cystic fibrosis
- Sickle anemia

Antisense and triplex DNA

- Antisense RNA or DNA
 - Prevent the synthesis of an unwanted protein
 - Targets mRNA
- Triplex DNA
 - Prevents transcription
 - Targets double stranded DNA

Genome Analysis

- Maps
- Fingerprints
- Family trees

Maps

- Determine the location of particular genes (locus) on the chromosome
- Determine differences in chromosomal regions (alleles)
 - Types of maps
 - Genomics and bioinformatics

Types of maps

- Linkage
 - Shows the relative proximity and location of genes
- Physical
 - Shows the proximity and size of genes
- Sequence
 - Shows the exact order of bases

Genomic and bioinformatics

- New discipline of study as a result of the enormous data generated by maps
 - Analyze and classify genes
 - Determine protein sequences
 - Determine the function of the genes

Fingerprinting

- Emphasizes the differences in the entire genome
- Techniques
 - Endonucleases
 - PCR
 - Southern blot
- Uses
 - Forensic medicine
 - Identify hereditary disease

Family trees

- Determine the genetic family tree for many diseases
- Pedigree of domestic animals
- Genetic diversity in animals