The Power of Molecular Biological Techniques

Professor

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Overview

I. Introduction to Molecular Pathology

II. DNA, Restriction Enzymes, Hybridization, PCR

III. Introduction to the Genome

IV. Applications to Molecular Medicine: SNPs and Chips

TEST YOUR SCIENCE LITERACY
Adapted from Dave Barry, Miami Herald

Explain in your own words, what is DNA?

1. **DNA is deoxyribonucleicantidisestablishmentarianism, a complex string of syllables found inside your body in tiny genes called chromosomes.**

2. **The information in your DNA determines your unique biological characteristics, such as eye color, Social Security number, and age. There is surprisingly little difference between DNA in humans, Democrats, and Republicans.**

Highly Sensitive and Specific

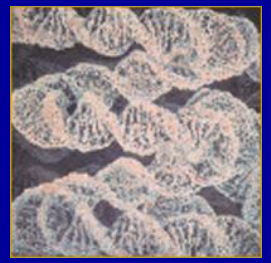
by "Orders of Magnitude"









BIOLOGY: THE STUDY OF LIFE

1. WHOLE ORGANISMS
2. ORGANS
3. TISSUES
4. CELLS
5. INTRACELLULAR ORGANELLES
6. CHEMICAL COMPONENTS

CHEMICAL COMPONENTS OF LIFE

1. PROTEINS
2. LIPIDS
3. NUCLEIC ACIDS:
   - DNA
   - RNA

MOLECULAR BIOLOGY TECHNIQUES

Molecular biology techniques utilize DNA, RNA, and enzymes that interact with nucleic acids to understand biology at a molecular level.

MOLECULAR PATHOLOGY

Molecular Pathology is a subspecialty of pathology that utilizes molecular biology techniques to:

- Detect normal and disease states (diagnosis)
- Predict disease progression (prognosis)

SUBSPECIALTIES OF MOLECULAR PATHOLOGY

- INHERITED DISEASES (GENETICS)
  - Cystic fibrosis
  - Sickle cell anemia
  - Predispositions to cancer
- INFECTIOUS DISEASES
  - Bacteria
  - Viruses
  - Fungi

SUBSPECIALTIES OF MOLECULAR PATHOLOGY

- HEMATOPATHOLOGY
  - Leukemias
  - Lymphomas
- SOLID TUMORS
  - Breast cancer
  - Colon cancer
  - Brain cancer

SUBSPECIALTIES OF MOLECULAR PATHOLOGY

- FORENSICS
- IDENTITY TESTING
  - HLA
  - parentage

NUCLEIC ACIDS

- Genetic material of all known organisms
- DNA: deoxyribonucleic acid
- RNA: ribonucleic acid (e.g., some viruses)
- Consist of chemically linked sequences of nucleotides
    - Nitrogenous base
    - Pentose- 5-carbon sugar (ribose or deoxyribose)
    - Phosphate group
- The sequence of bases provides the genetic information

Bases

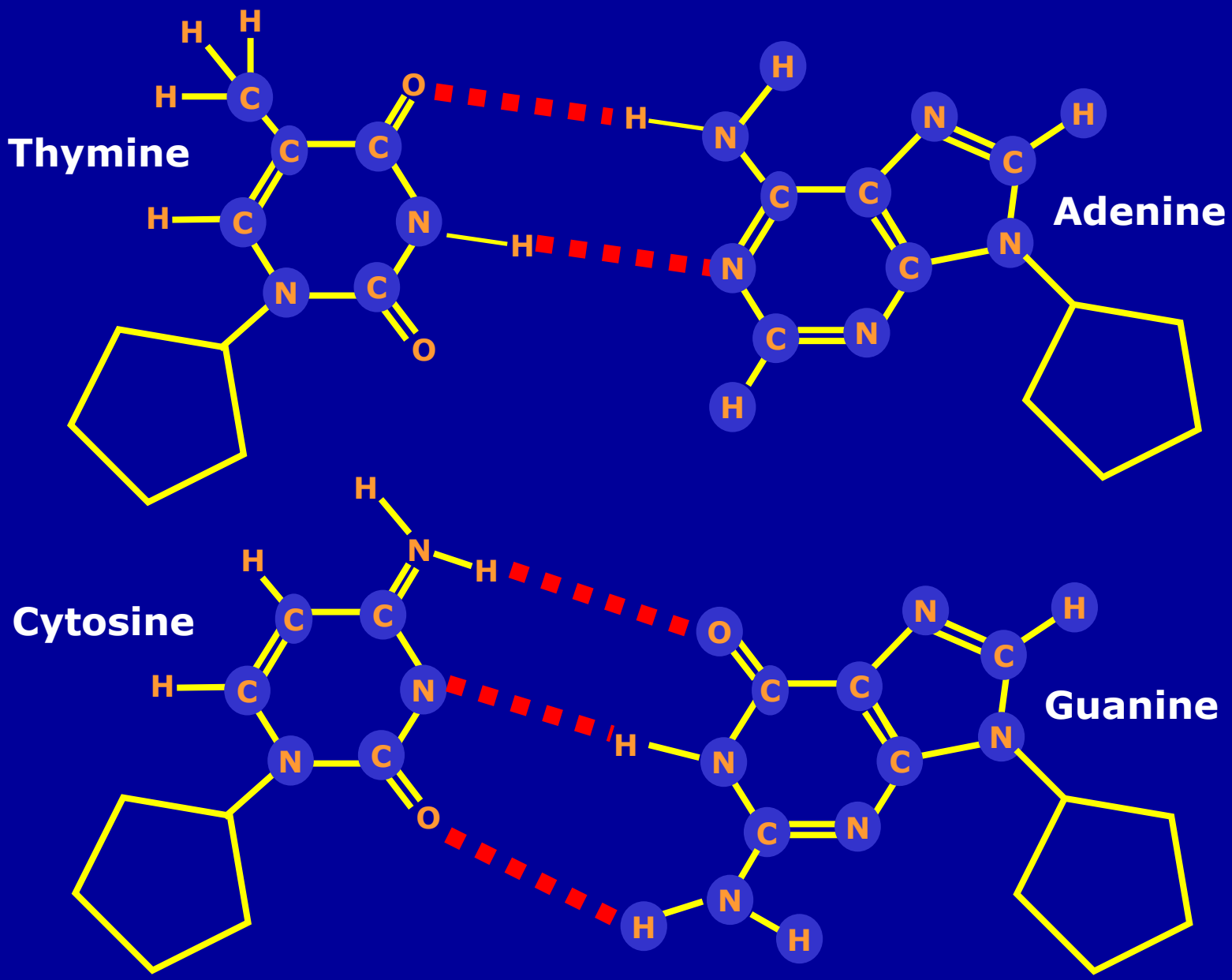
- Two types of bases
- Purines are fused five- and six-membered rings
  - Adenine A DNA RNA
  - Guanine G DNA RNA
- Pyrimidines are six-membered rings
  - Cytosine C DNA RNA
  - Thymine T DNA
  - Uracil U RNA

Base-pairing

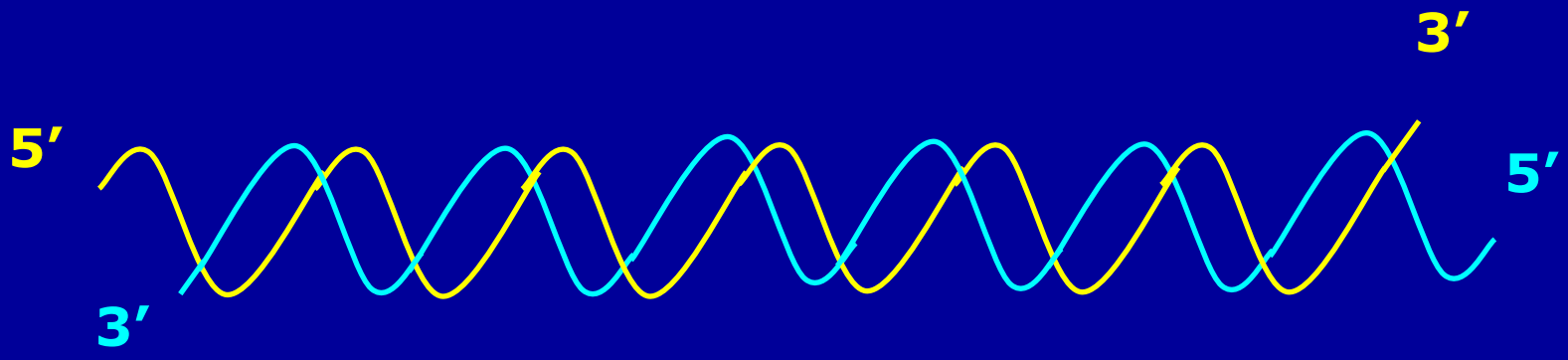
- Hydrogen bonds are relatively weak bonds compared to covalent bonds
- Hydrogen bonds can form between a pyrimidine and a purine

- Watson-Crick base-pairing rules
  - A═T
  - G≡C

Hydrogen Bonds



DNA: Helix

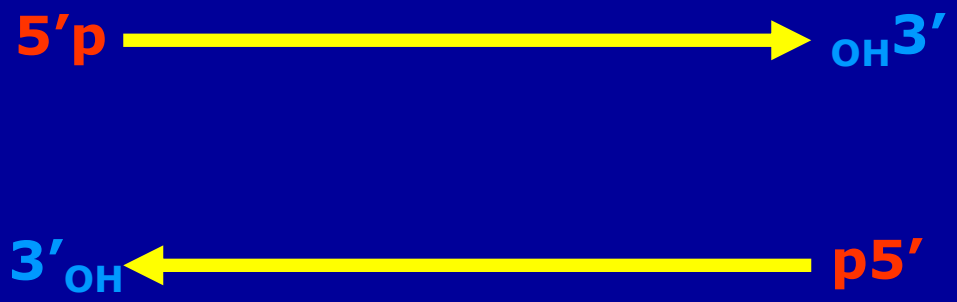


In general, DNA is double-stranded. Double-stranded (ds) DNA takes the form of a right handed helix with approximately 10 base pairs per turn of the helix.

Complementarity

- In the DNA double helix, purines and pyrimidines face each other
- The two polynucleotide chains in the double helix are connected by hydrogen bonds between the bases
- Watson-Crick base-pairing rules
    - A═T
    - G≡C
- GC base pairs (bps)have more energy than AT bps
- Since one strand of DNA is complementary to the other, genetic material can be accurately reproduced; each strand serves as the template for the synthesis of the other

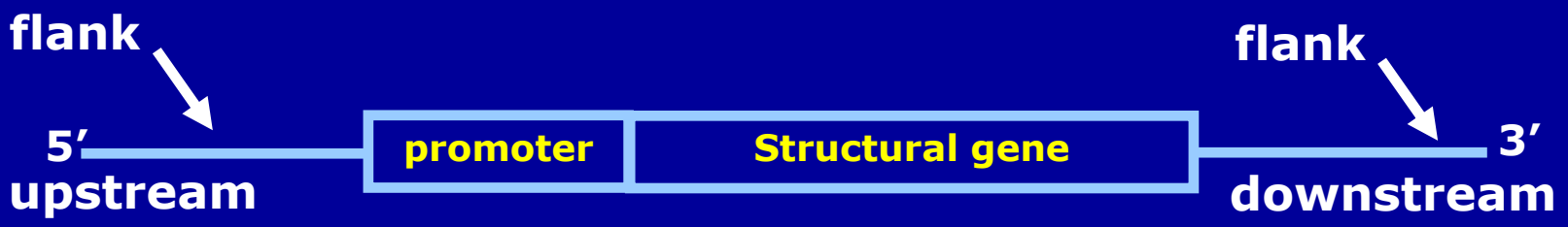
Antiparallel Chains



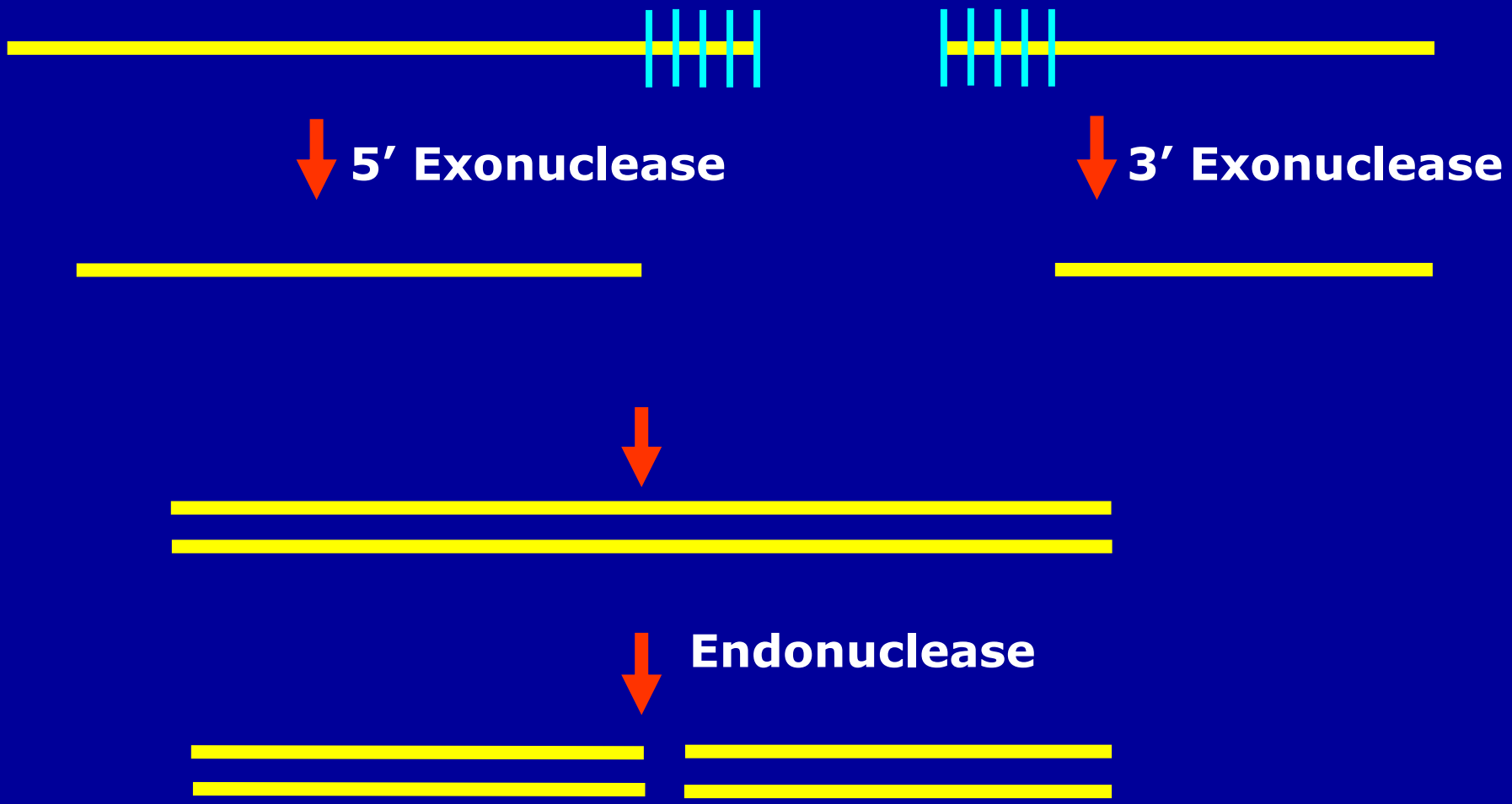
Two strands of the DNA double helix are antiparallel and complementary to each other

Gene

- A gene is a unit of inheritance
- Carries the information for a:
  - -polypeptide
  - -structural RNA molecule



Nucleases



Restriction enzymes

- **Specific endonucleases**
- **Recognize specific short sequences of DNA and cleave the DNA at or near the recognition sequence**
- **Recognition sequences: usually 4 or 6 bases but there are some that are 5, 8, or longer**
- **Recognition sequences are palindromes**
- **Palindrome: sequence of DNA that is the same when one strand is read from left to right or the other strand is read from right to left– consists of adjacent inverted repeats**

Restriction enzymes (cont'd)

- **Example of a palindrome:**

  GAATTC

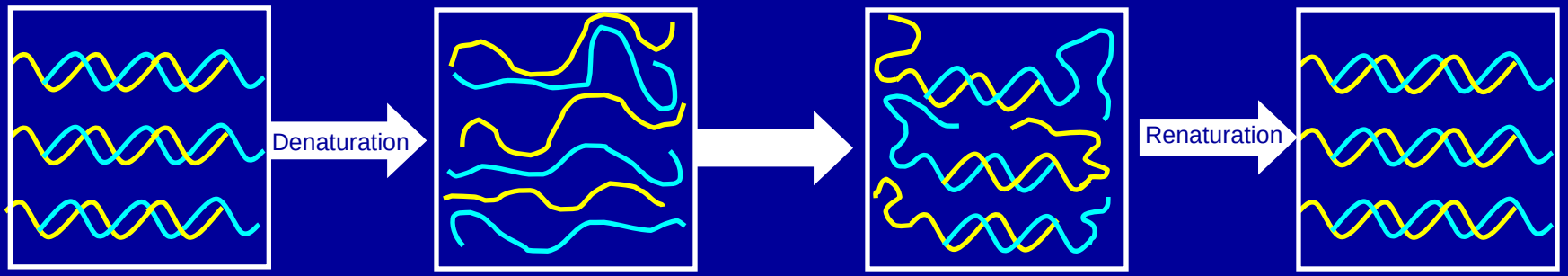
  CTTAAG

- **Restriction enzymes are isolated from bacteria**
  - **Derive names from the bacteria**
  - **Genus- first letter capitalized**
  - **Species- second and third letters (small case)**
  - **Additional letters from "strains"**
  - **Roman numeral designates different enzymes from the same bacterial strain, in numerical order of discovery**
  - **Example: EcoRI**
    - **E *Escherichia***
    - **Co *coli***
    - **R R strain**
    - **I first enzyme discovered from *Escherichia coli* R**

Hybridization

- Nucleic acid hybridization is the formation of a duplex between two complementary sequences
- Intermolecular hybridization: between two polynucleotide chains which have complementary bases
  - DNA-DNA
  - DNA-RNA
  - RNA-RNA
- Annealing is another term used to describe the hybridization of two complementary molecules

Denaturation - Renaturation



Double-stranded DNA

Single-stranded DNA

Initial Base pairing

Renatured DNA

Probes

- **Probe is a nucleic acid that**
  - **can be labeled with a marker which allows identification and quantitation**
  - **will hybridize to another nucleic acid on the basis of base complementarity**
- **Types of labels**
  - **Radioactive (^{32}P, ^{35}S, ^{14}C, ^{3}H)**
  - **Fluorescent**
    - **FISH: fluorescent in situ hybridization**
      - **chromosomes**
  - **Biotinylated (avidin-streptavidin)**

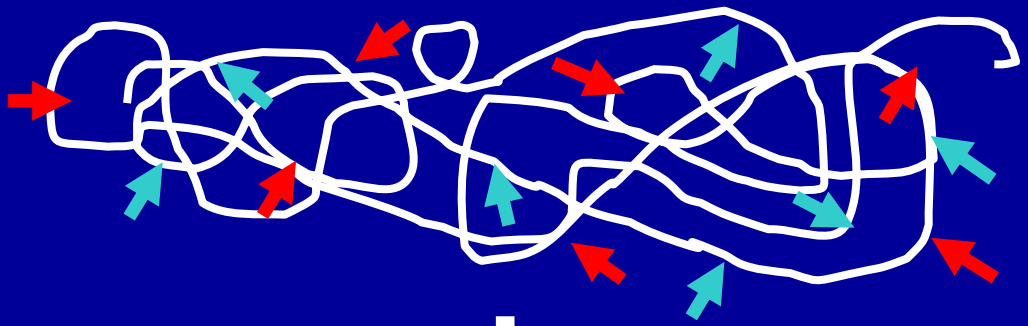
Solid Support Hybridization

- **Solid support hybridization: DNA or RNA is immobilized on an inert support so that self-annealing is prevented**
- **Bound sequences are available for hybridization with an added nucleic acid (probe).**
- **Filter hybridization is the most common application:**
  - **Southern Blots**
  - **Dot/Slot Blots**
  - **Northern Blots**
- **In-silica hybridization (glass slides)**
  - **in situ hybridization (tissue)**
  - **Chromosomal (FISH)**
  - **Microarrays**

Southern Blots

- Southern blotting is a procedure for transferring denatured DNA from an agarose gel to a solid support filter where it can be hybridized with a complementary nucleic acid probe
- The DNA is separated by size so that specific fragments can be identified
- Procedure:
  - Restriction digest to make different sized fragments
  - Agarose gel electrophoresis to separate by size
  - Since only single strands bind to the filter, the DNA must be denatured.
  - Denaturation to permit binding to the filter (NaOH)
  - Transfer to filter paper (capillary flow)
  - Hybridization to probe
  - Visualization of probe

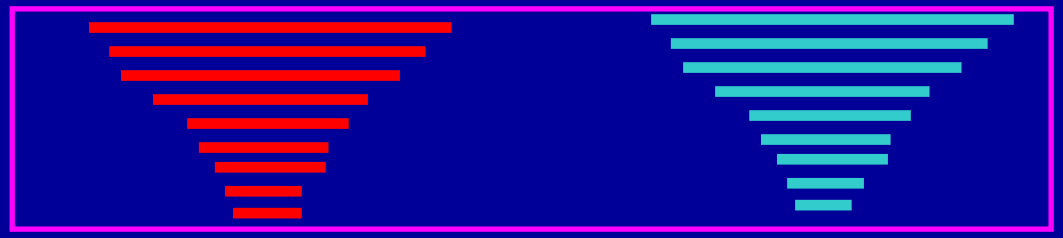
Southern Blot



Restriction enzyme

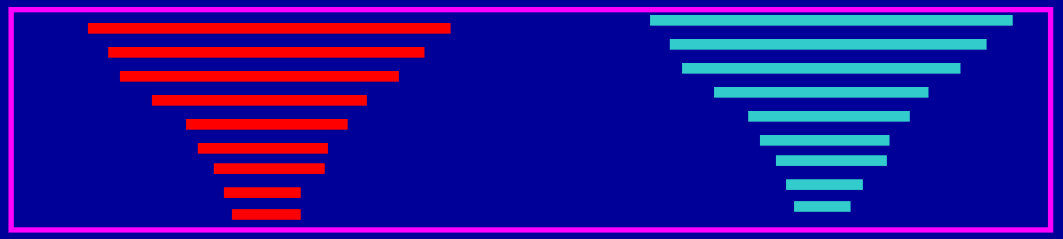
DNA of various sizes

Electrophorese on agarose gel



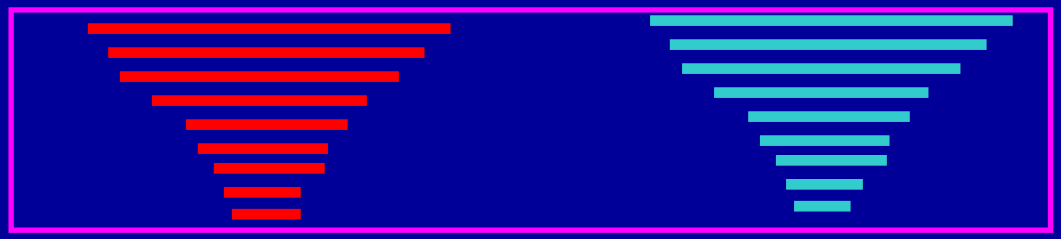
gel

Denature - transfer to filter paper.



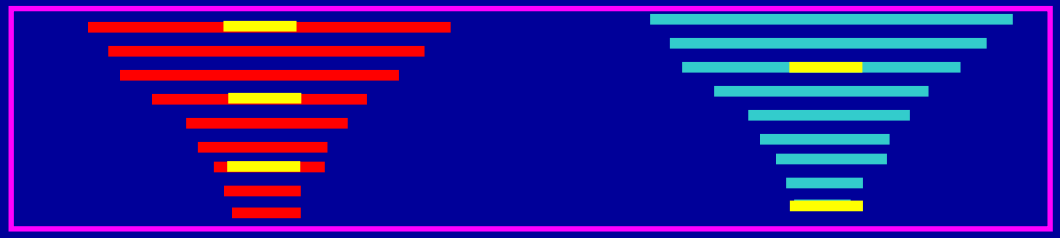
blot

Denature- transfer to filter paper.

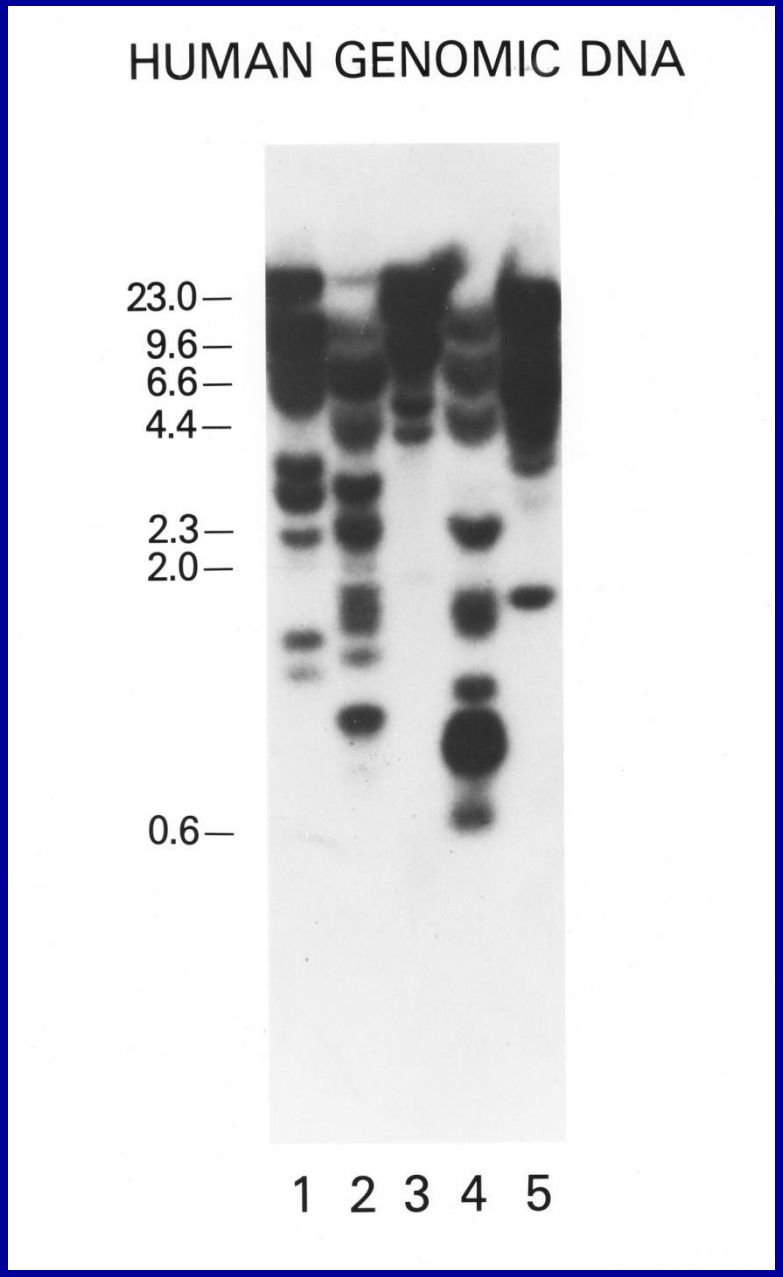


blot

Hybridize to probe



Visualize

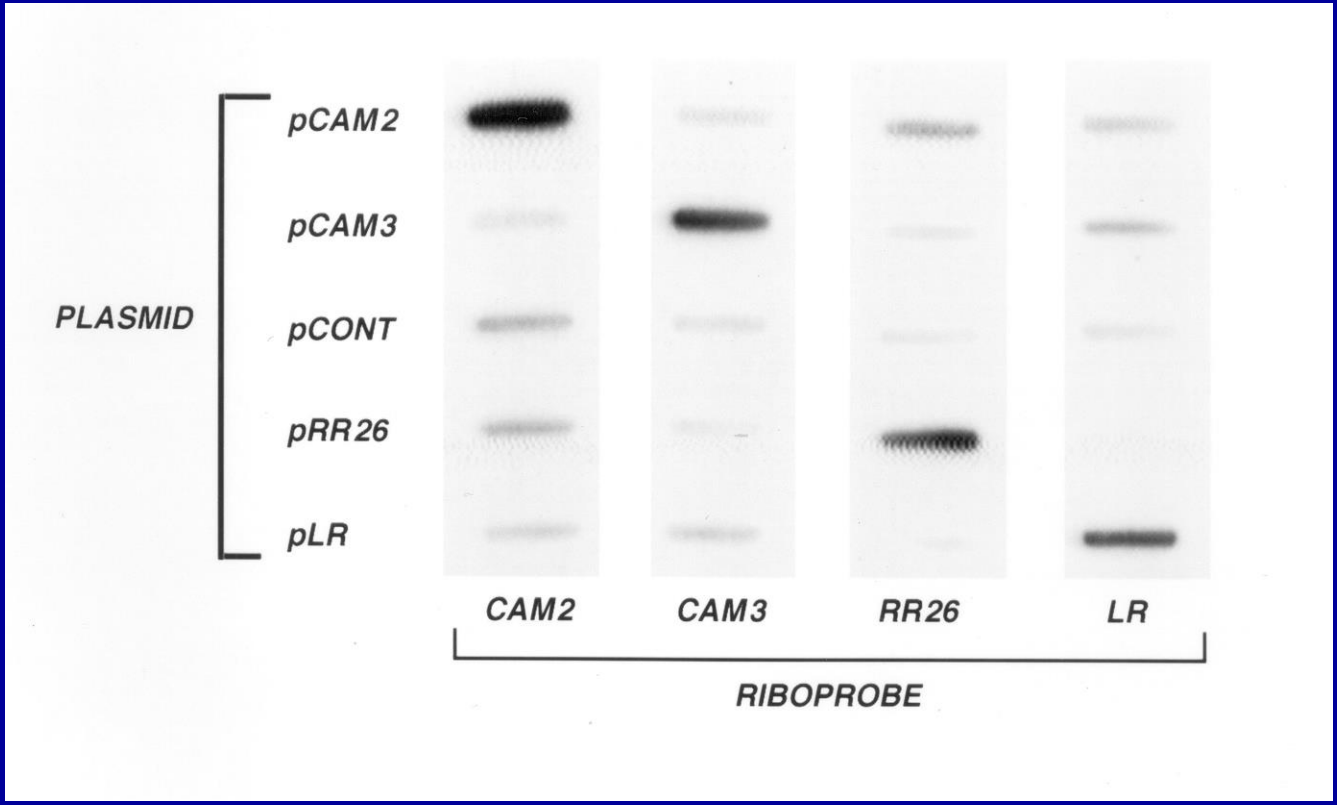


Southern Blot

Dot/Slot Blots

- DNA or RNA is bound directly to a solid support filter
- No size separation
- Ideal for multiple samples and quantitative measurements
- Important to establish specificity of conditions

Slot Blot

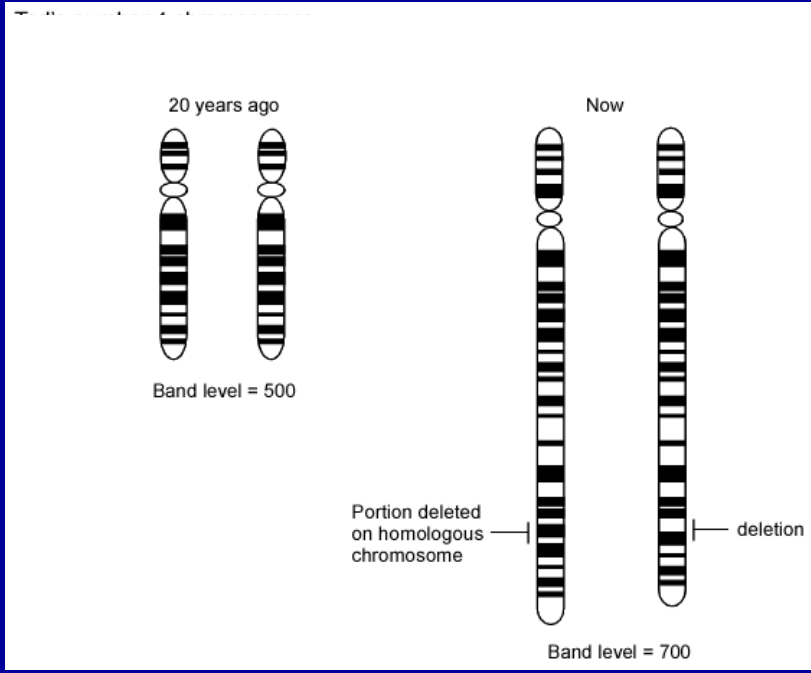

PLASMID
pCAM2
pCAM3
pCONT
pRR26
pLR
CAM2
CAM3
RR26
LR
RIBOPROBE

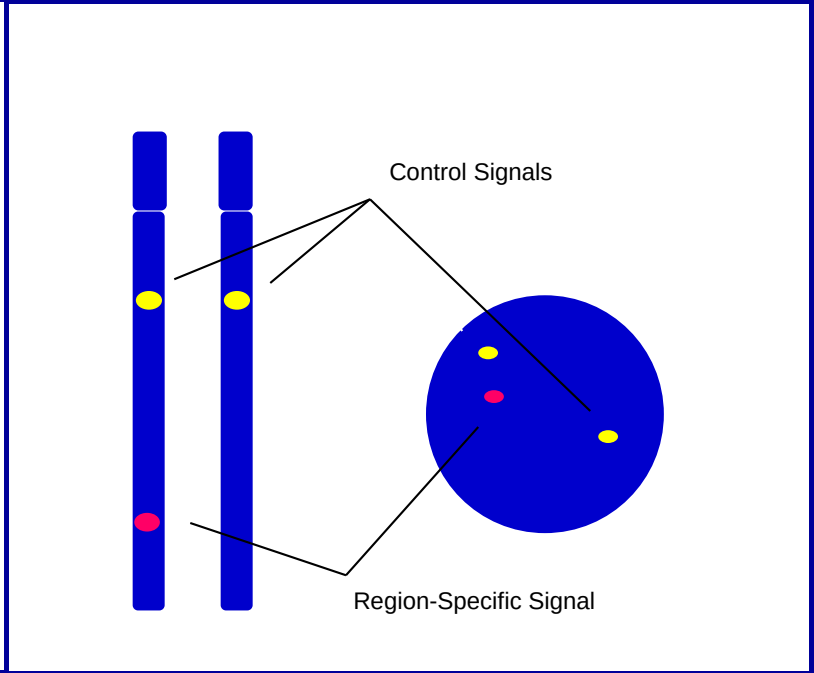
A Focus of Development: Automation

User-Friendly, Faster, and Cost-Effective

This electronic microarray is an example of "Lab-on-a-Chip" technology. It is an electrophoresis device that produces results up to 1000 times faster than conventional techniques while using much less sample.

High Resolution Banding and FISH





The chromosome banding technique performed 20 years ago missed the small deletion. High resolution banding developed more recently can elucidate the abnormality. Fluorescence *in situ* Hybridization (FISH) is a powerful technique in that it can reveal submicroscopic abnormalities even in non-dividing cells.

Polymerase chain reaction

- PCR is the in vitro enzymatic synthesis and amplification of specific DNA sequences
- Can amplify one molecule of DNA into billions of copies in a few hours

Applications of PCR

- Detection of chromosomal translocations
  - Amplification across a translocation sequence
- Chromosome painting
- Detection of residual disease
- Infectious disease
- Forensics
- HLA typing
- Detection of Loss of Suppressor Genes
  - Loss of Heterozygosity (LOH)

Genome Literacy

- **Genome: The entire DNA of an organism**
  - **Humans**
    - **diploid (chromosome pairs)**
    - **6 x 10^9 bp per diploid genome**
    - **Haploid genome is one set of chromosomes**
- **Chromosome: structure found within a cell nucleus consisting of a continuous length of ds DNA**
  - **Humans**
    - **22 pairs of autosomal chromosomes**
    - **2 sex chromosomes**

Human Genome Project

- 40,000 genes
- Speaking a language of molecular fingerprints
- Gene expression is another language of complexity

Genome Mapping Terms

- Locus: a position on a chromosome
- Allele: alternate form of DNA at a specific locus on the chromosome
  - Each individual inherits two copies of DNA
    - Maternal
    - Paternal
  - Homozygous alleles: the two copies are identical
  - Heterozygous alleles: the two copies are different

Restriction fragment length polymorphism

- RFLP is a polymorphic allele identified by the presence or absence of a specific restriction endonuclease recognition site:
  - GAATTC versus GATTTC
- RFLP is usually identified by digestion of genomic DNA with specific restriction enzymes followed by Southern blotting
- Regions of DNA with polymorphisms:
  - Introns
  - Flanking sequences
  - Exons

Genetic Variation

- **Most genes have small sequence differences between individuals**
  - **Occur every 1350 bp on average**
- **Some of these polymorphisms may affect:**
  - **How well the protein works**
  - **How the protein interacts with another protein or substrate**
- **The different gene forms containing polymorphisms are called alleles**

Mutation detection

- Sequence DNA
- Hybridization Methods
  - Blotting
  - Chips
- Restriction enzyme polymorphisms:
  - GAATTC versus GATTTC
- SNPs (single nucleotide polymorphisms)

SNPs

- Single nucleotide polymorphisms
- Distinction from mutations

ASO

Allele Specific Oligonucleotides

ATGTGGCCATGTGGC

ATGCGGCCATGTGGC

ASOs can be used to detect SNPs

(single nucleotide polymorphisms)

More About SNPs

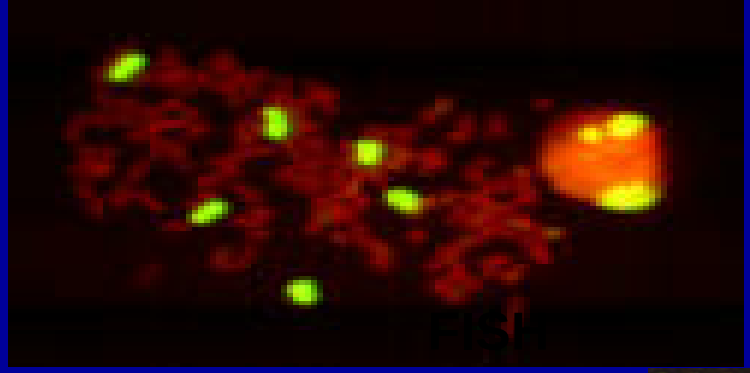
- **SNPs in exons are called coding SNPs**
- **SNPs in introns or regulatory regions may affect transcription, translation, RNA stability, RNA splicing**

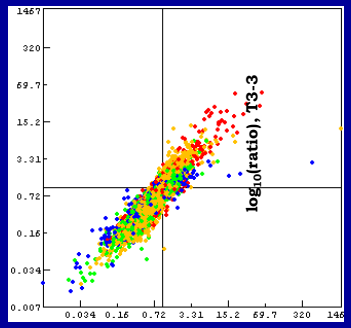
Pharmacogenomics

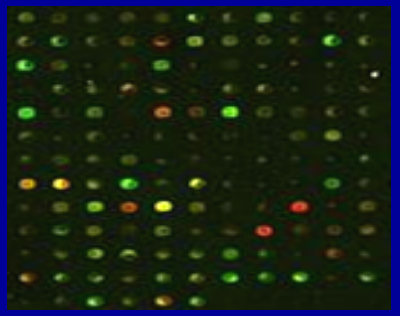
- Cytochrome P450
- Uptake and metabolsim of drugs
- Seizure disorders
- Psychiatric disorders
- Cancer therapy

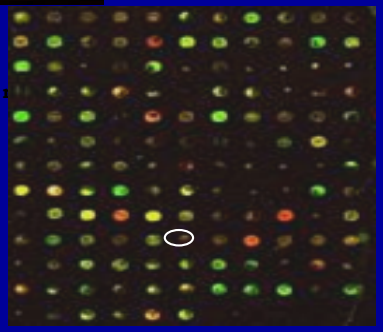
Resources

- www.amptestdirectory.org is an online directory of laboratories that perform molecular techniques.
- www.genetests.org has an illustrated glossary and good explanations of genetic testing.
- http://www.ornl.gov/sci/techresources/Human_Genome/education/images.shtml has links to many educational resources and images.
- http://www.dnalc.org/resources/resources.html has an animated DNA primer targeted at the level of a "bright teenager." It is a part of the website of the Dolan DNA Learning Center of Cold Spring Harbor Laboratory.









Microarrays

genome

Laser microscope

Tissue arrays