Medical Biotechnology

The Power of Molecular Biology: Detecting and Diagnosing Human Disease Conditions

- Models of Human Disease
  - A number of human genetic diseases also occur in model organisms
  - Can therefore use model organisms to identify disease genes and **test gene therapy** and **drug-based therapeutic approaches** to check their effectiveness and safety in preclinical studies

- Models of Human Disease
  - Extremely important because we cannot manipulate human genetics for experimental purposes
  - Many genes in different species have been shown to be similar to human genes based on DNA sequence – called **homologs**

Obese mice
lacks *Ob* gene

Normal mice
with *Ob* gene

Ob gene encodes a protein hormone leptin, which is produced by fat cells and travels through the bloodstream to the brain to regulate hunger.

Human homologue for *Ob* gene is discovered – can provide insights into fat metabolism in humans and genetics for weight disorders

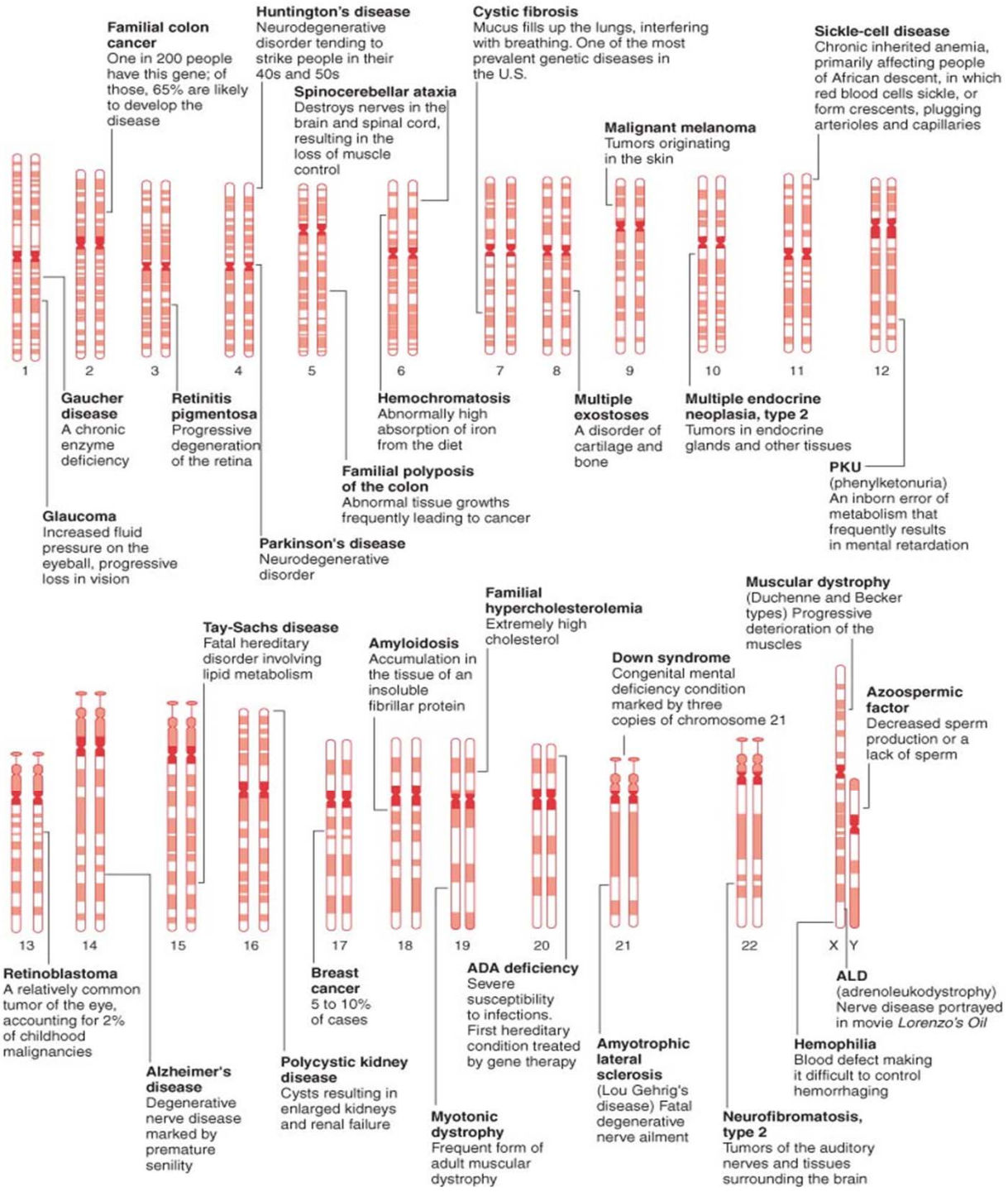
- In developing embryo, some cells must die to make room for others. How does the body know which cells develop into organs and which should die?
- Embryo development model – *C. elegans*, unsegmented roundworm
- Lineage of all cells in the embryonic worm that develop to form a mature organ is traced
- Some cells die by cell suicide known as programmed cell death, or **apoptosis**.
- Apoptosis is involved in neurodegenerative diseases (Alzheihmer, Huntington, Parkinson diseases), arthritis

Similarity of Humans with others

% of Genes Similar	Organism
31	Yeast
40	Roundworms
50	Fruit Fly
90	Mice

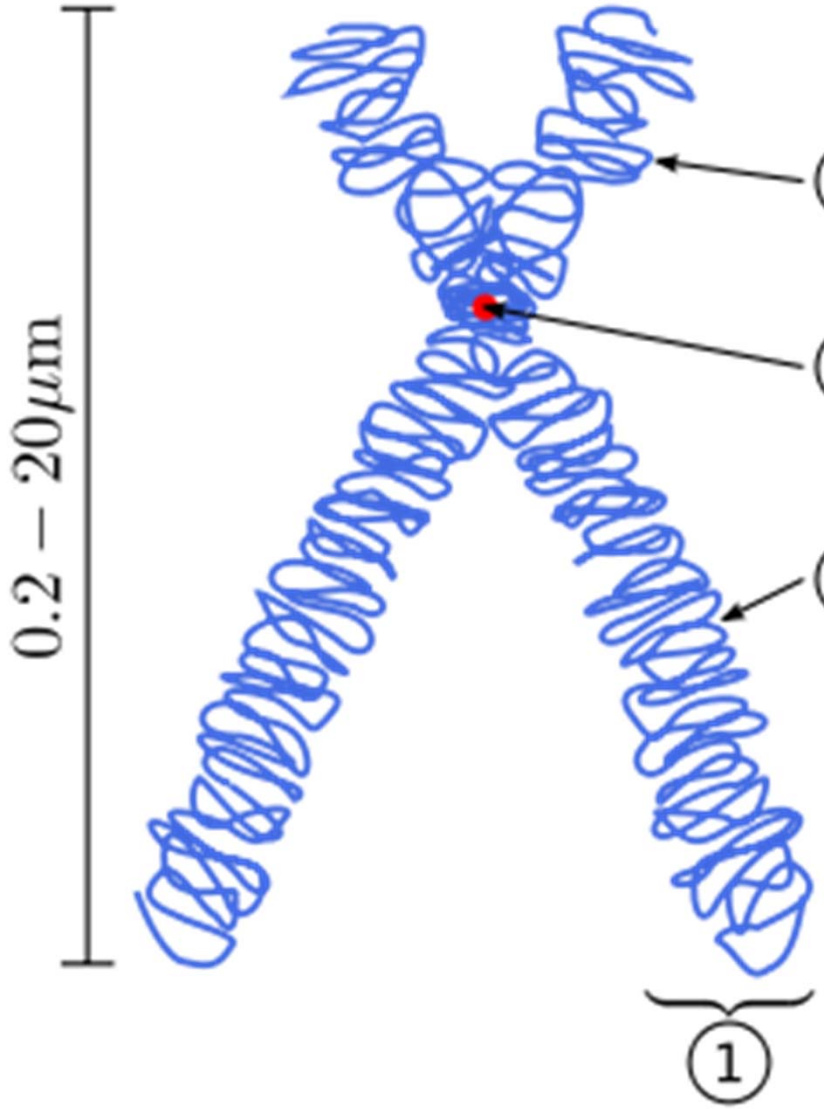
- Biomarkers for Disease Detection
  - Early detection of disease is critical for providing the best treatment and improving the odds of survival
  - With the right diagnostic tools, may be possible to detect most every disease at an early stage
  - **Biomarkers** – typically proteins produced by diseased tissue or proteins whose production is increased when a tissue is diseased
    - PSA, prostate-specific antigen

Disease Genes on Human Chromosomes



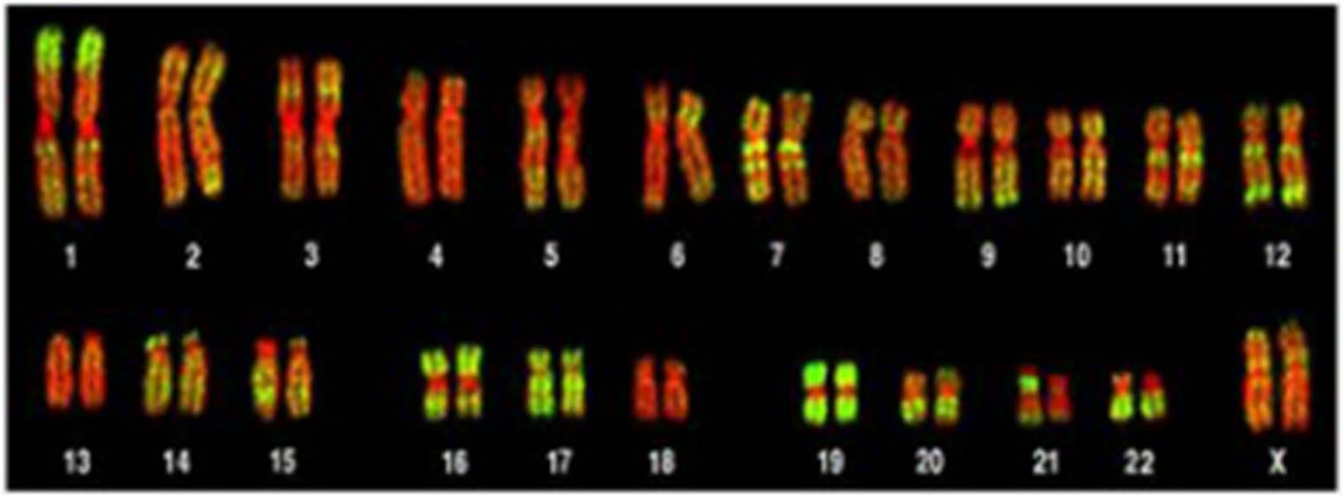
- Detecting Genetic Diseases
  - Down Syndrome: 3 copies of chr 21 (trisomy 21)
  - Mouse model is created with almost complete copy of human Chr21
  - Fetal testing for Down Syndrome may be done
  - Testing for chromosome abnormalities can be done by creating a karyotype

Eukaryotic Chromosome

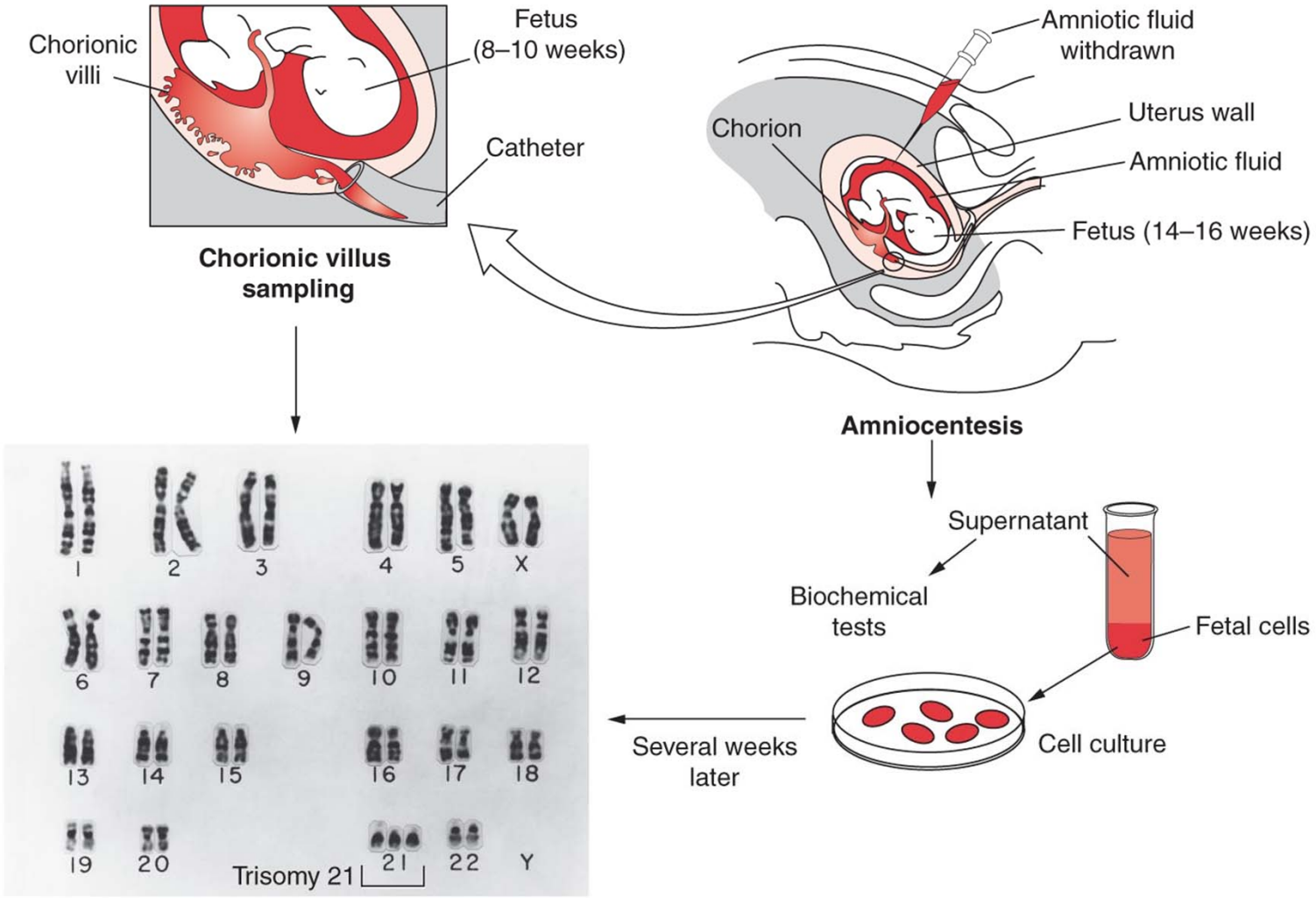


Karyotypes

- A karyotype is an organized profile of an individual's chromosomes
- The chromosomes are stained with different dyes that bind to proteins attached to the DNA, creating patterns of light and dark bands on each chromosome.
- In a karyotype, the chromosomes are arranged and numbered by size, from largest to smallest, the position of the centromere, and according to the characteristic pattern of their bands.

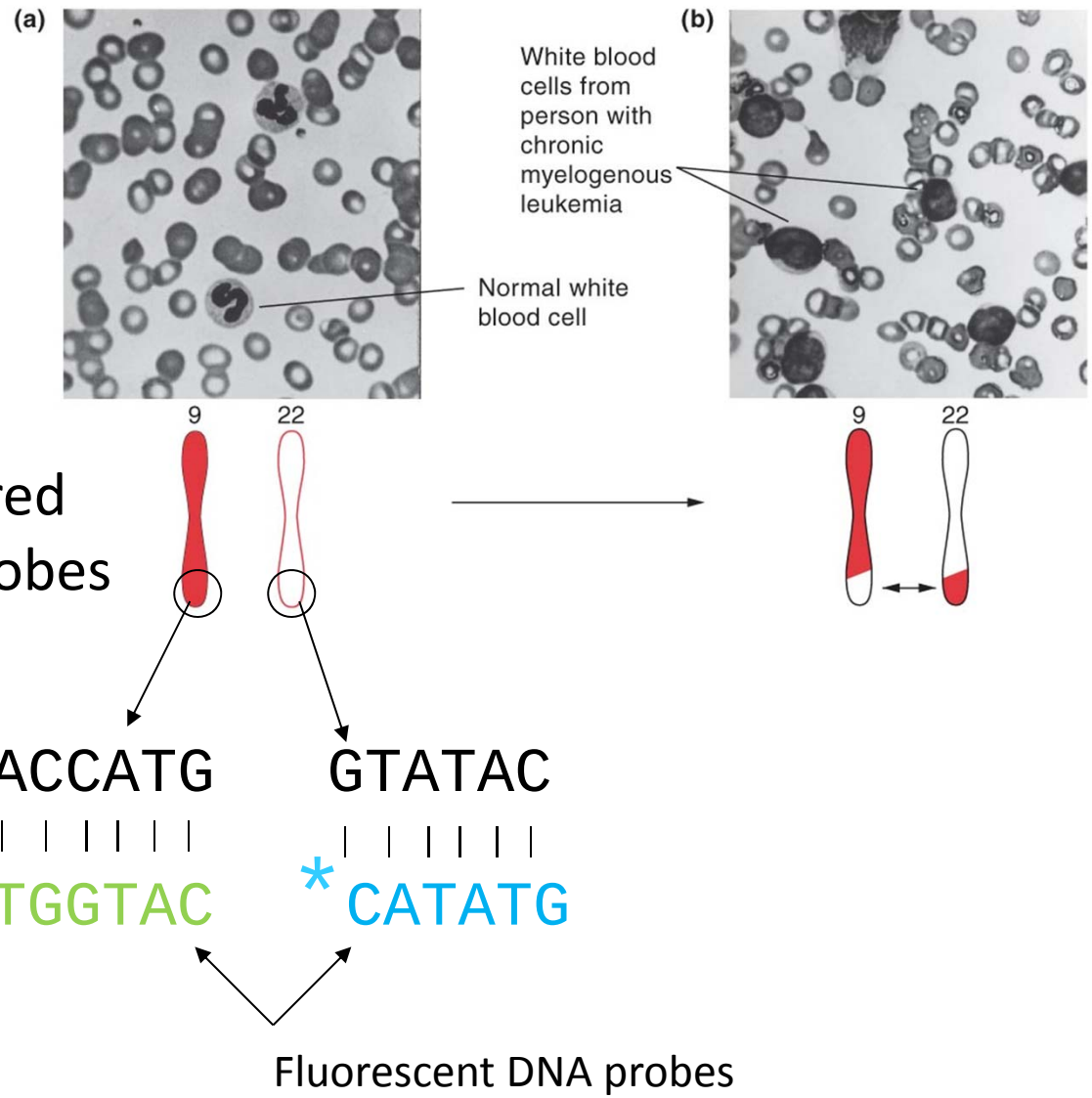


Detecting Genetic Diseases: Testing for chromosome abnormalities

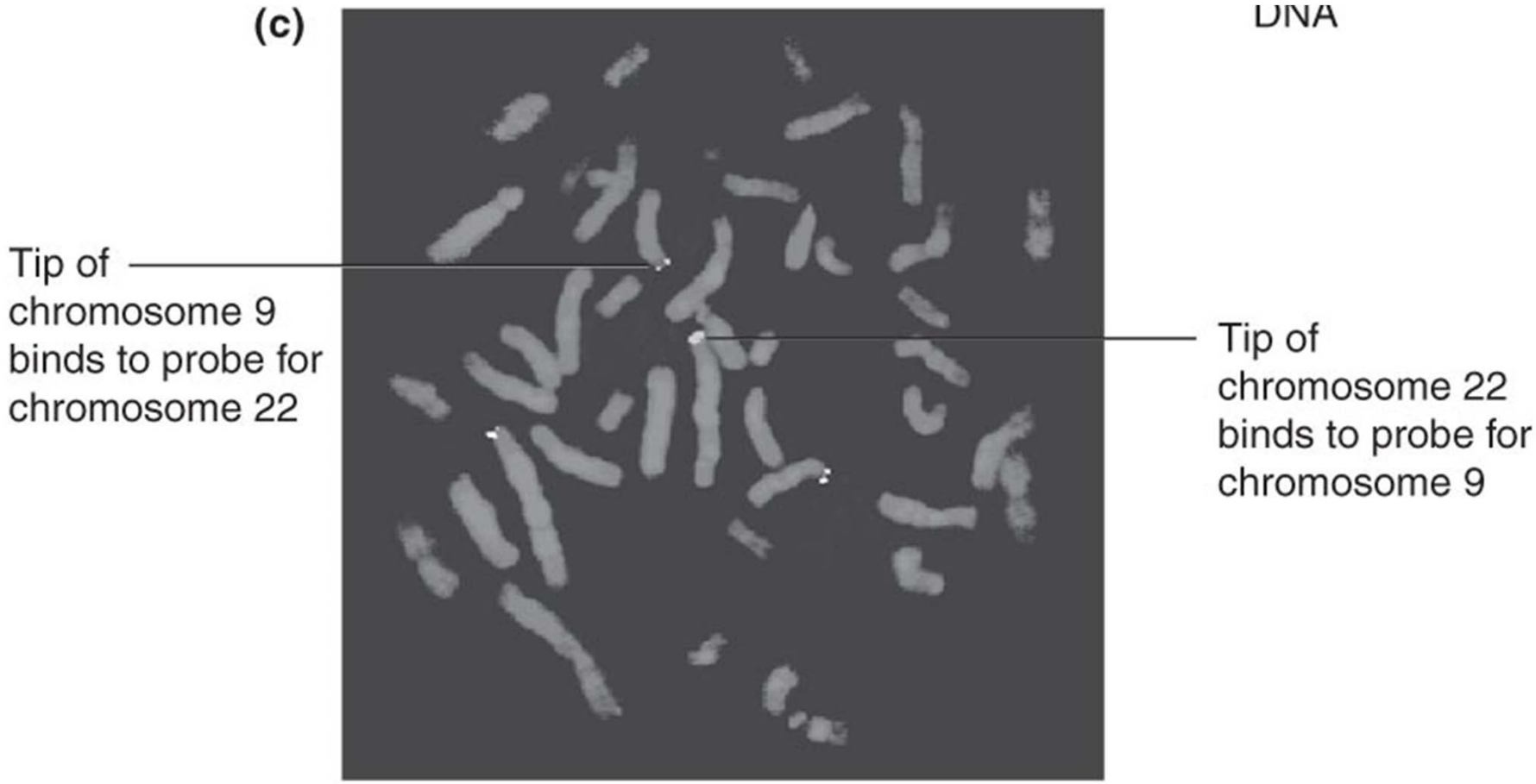


- Detecting Genetic Diseases
  - Testing for chromosome abnormalities
    - Fluorescence *in situ* hybridization (FISH) – new technique for karyotyping
      - Chromosome spread is prepared on a slide and then fluorescent probes (specific for certain marker sequences) are hybridized to each chromosome
      - Useful for identifying missing chromosomes and extra chromosomes, but much easier to detect defective chromosomes
      - Chromosomal deletion, chromosomal swapping due to problems in replication

FISH detection of Chronic myelogenous leukemia

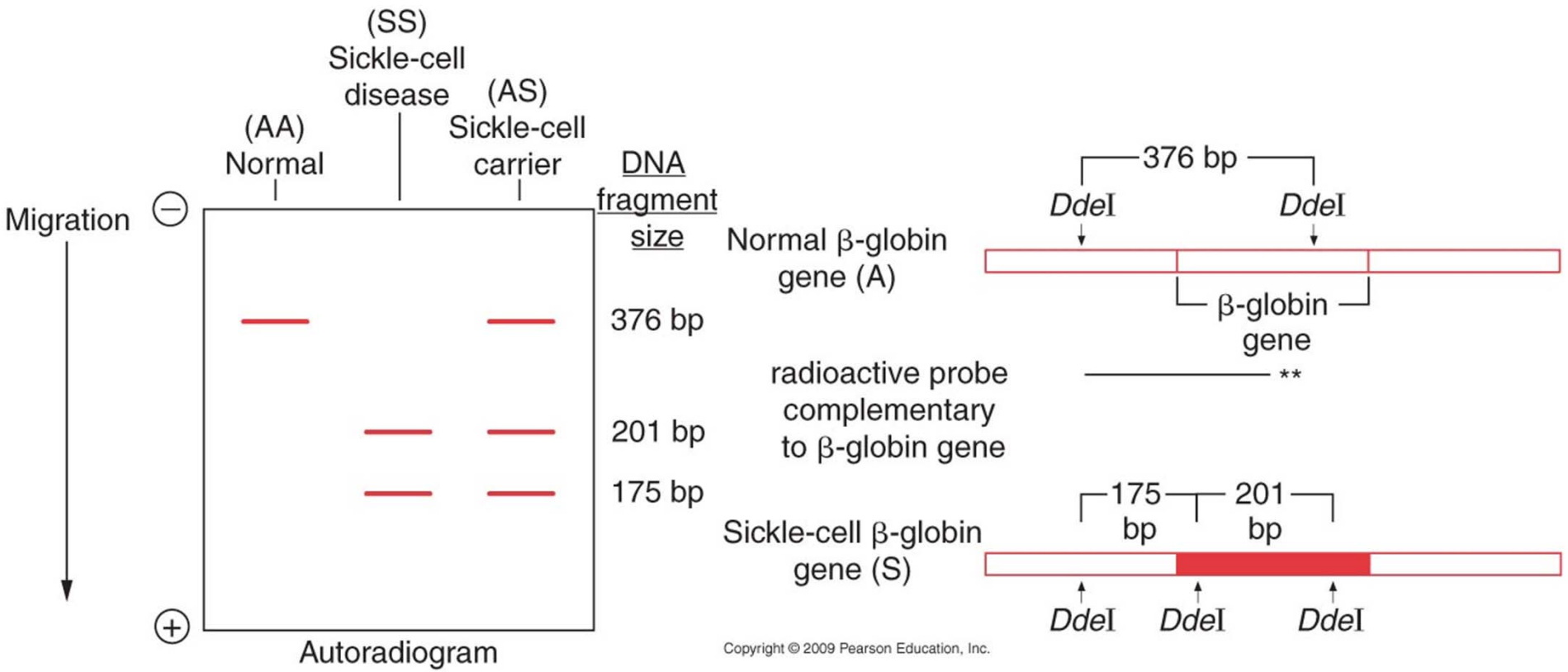


Different colored fluorescent probes are used


(c)
DNA
Tip of chromosome 9 binds to probe for chromosome 22
Tip of chromosome 22 binds to probe for chromosome 9

- Detecting Genetic Diseases
  - Most genetic diseases result from mutations in specific genes
    - RFLP (Restriction Fragment Length Polymorphism)
    - Defective gene sequences may be cut differently by restriction enzymes than their normal complements because nucleotide changes in the mutant genes can affect restriction enzyme cutting sites
    - Sickle-cell disease can be detected – DNA subjected to restriction digest, Southern blot analysis with a probe for β-globin gene is performed

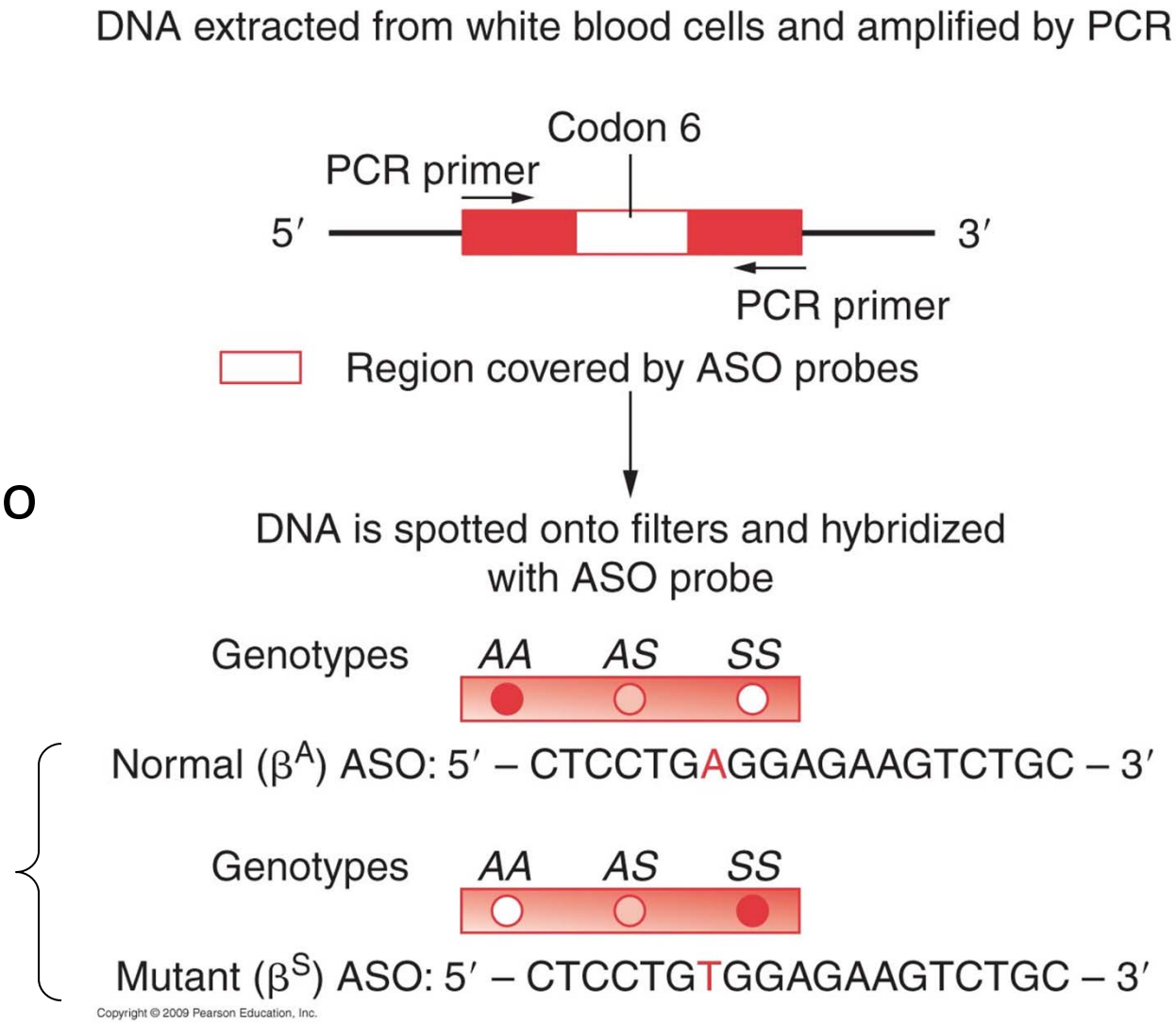
RFLP analysis to detect Sickle cell disease



- Detecting Genetic Diseases
  - Most genetic diseases result from mutations in specific genes
    - RFLP is limited in that it can only be used if mutation changes a restriction site in a gene
    - **Allele-specific oligonucleotide analysis** (ASO) – allows for the detection of single nucleotide changes even if the mutation does not change a restriction site
      - DNA is isolated from human cells, and then amplified by PCR using primers that flank gene of interest.
      - Amplified DNA is blotted onto nylon membrane and hybridized with two different ASO probes.
      - ASOs are small (20 bps), single-stranded oligonucleotide sequences.

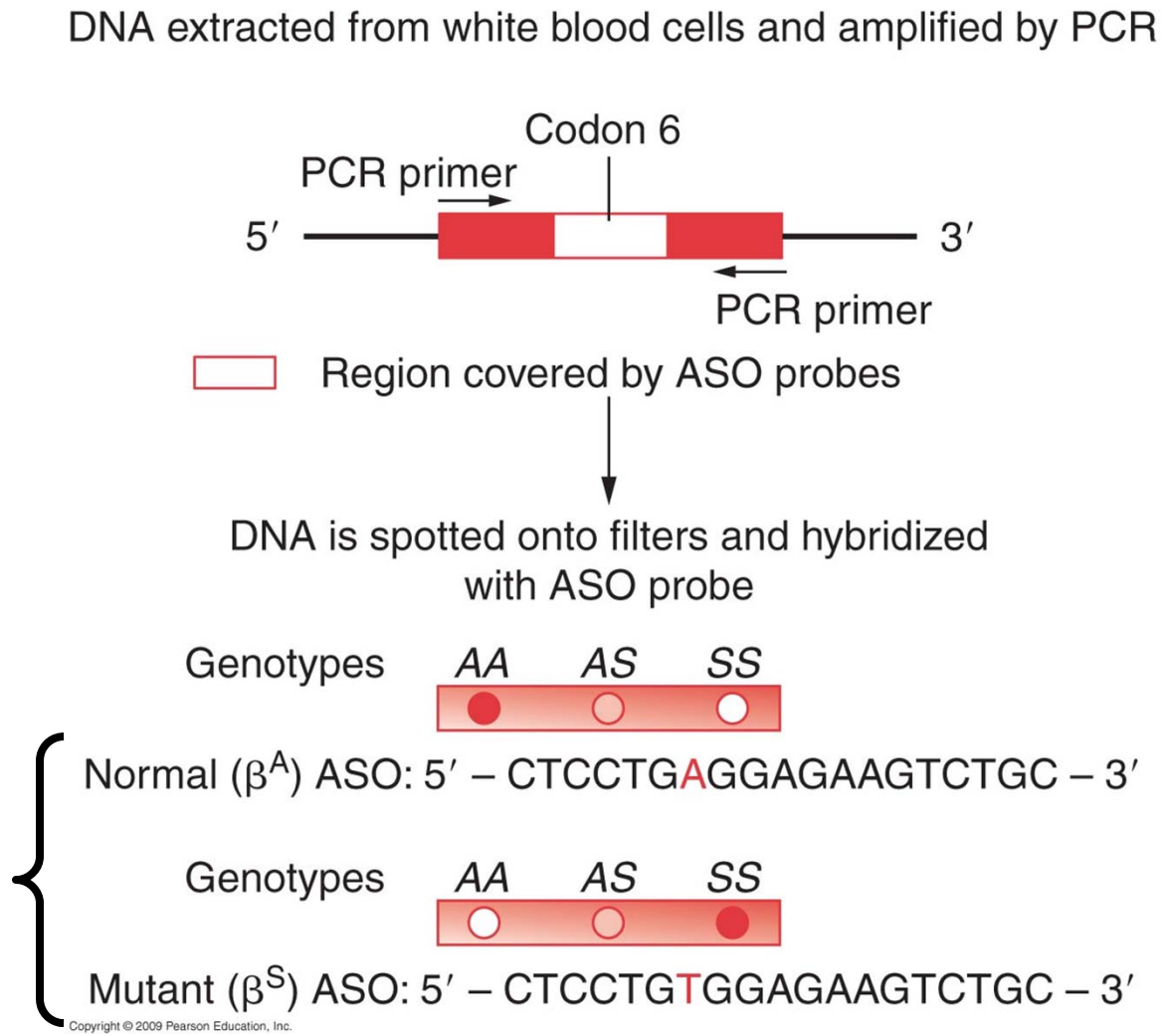
Allele specific oligonucleotide analysis (ASO)

- Analyze DNA from cells of 8-32-cell-stage-old embryo created by in vitro fertilization
- Allows individuals to select health embryos before implantation



Allele specific oligonucleotide analysis (ASO)

Oligonucleotide probes that react with sequence of normal β^A gene or disease β^S gene

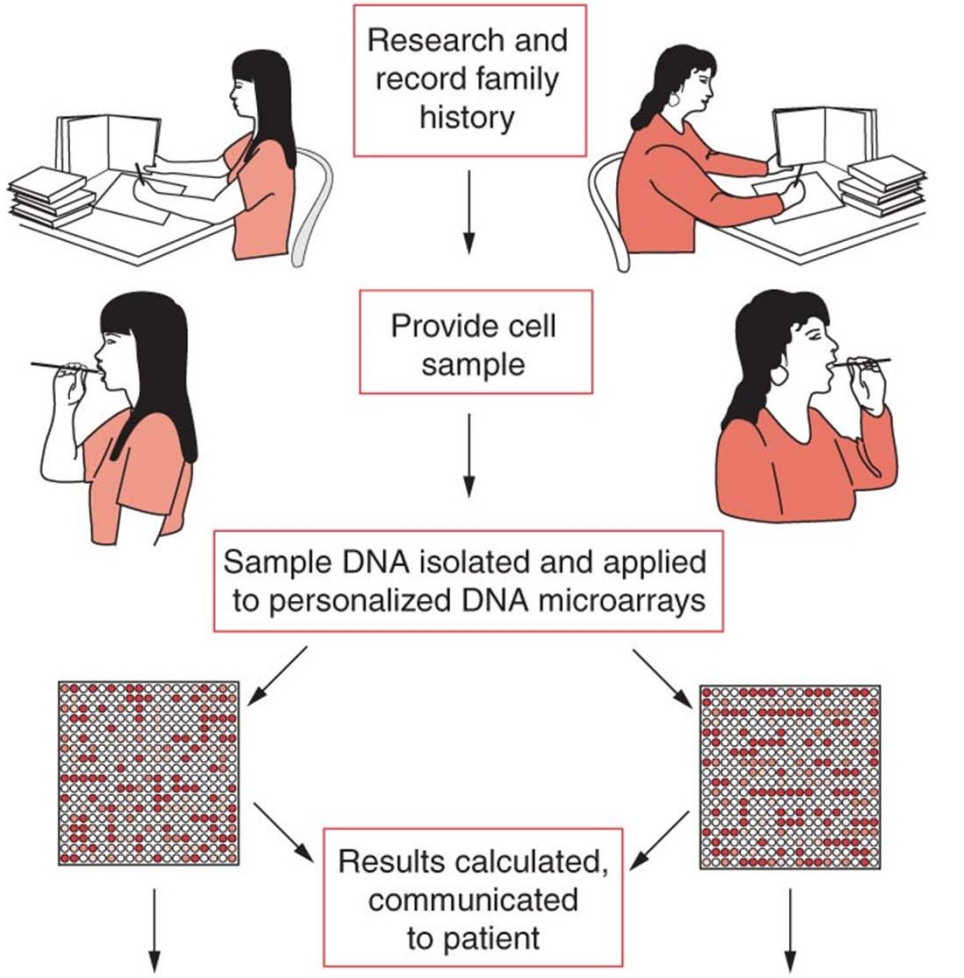


SNPs are abundant

- **SNPs** (single nucleotide polymorphisms)
  - Estimated that 1 SNP occurs every 1000-3000 bp along the DNA of every chromosome
  - Over 1.4 million SNPS identified to date on human chromosome
  - One of the most common forms of genetic variation among humans
  - If an SNP occurs in a gene sequence, it may cause a change in protein structure that produces disease or influences traits in a variety of ways
  - **HapMap** project: Pharmaceutical companies, academic institutions, and private foundations are together cataloguing the chromosomal locations of all known SNPs
  - Might be used to predict susceptibilities to
    - Stroke, diabetes, cancer, heart disease, behavioral and emotional illnesses

- Detecting Genetic Diseases
  - **DNA microarrays** are glass microscope slides spotted with thousands of genes
    - Can be used to screen a patient for a pattern of genes that might be expressed in a particular disease condition

Identifying sets of disease genes by microarrays



Susan's Genetic Profile

Trait	Risk
Addictive behavior	: Greater than general population
Lung cancer	: Greater than general population
Colon cancer	: Less than general population
Alzheimer's disease	: Less than general population

Lisa's Genetic Profile

Trait	Risk
Cystic fibrosis	: 100% diagnosis
Type II diabetes mellitus	: Less than general population
Cardiovascular disease	: Greater than general population