Chapter 9-Molecular Diagnostics

ELISA
Monoclonal Antibodies
DNA Diagnostic Systems (DNA fingerprinting)
Molecular Diagnosis of Genetic Disease

Old vs. New Molecular Diagnostics

- Old: grow cells/pathogen->test
- Such growth can be a problem as it is sometimes slow, costly, and specific
- New: direct test (either immunological or DNA based)
- Detection must be: specific, sensitive, and simple (fast is also nice)

Fig. 9.1 Enzyme-Linked Immunosorbent Assay (ELISA): immunological detection

A. Bind sample to the support (commonly plastic or a membrane)
B. Add primary antibody; wash
C. Add secondary antibody-enzyme conjugate; wash
D. Add substrate

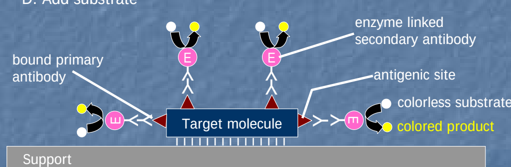
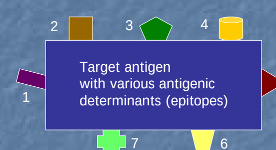


Fig. 9.2 Target antigens and polyclonal versus monoclonal antibodies



Polyclonal antibodies are made against and react with multiple antigenic sites (epitopes) on a target antigen. Monoclonal antibodies are directed against a particular antigenic site.

Fig. 9.4 Procedure for producing a monoclonal antibody to protein X

Note: B lymphocytes or B cells produce antibodies but do not reproduce in culture. Some B cells can become cancerous and are known as myelomas which can reproduce in culture.

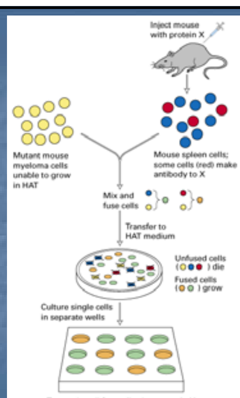
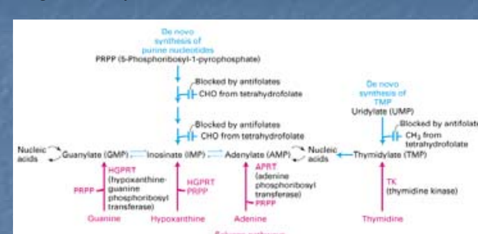


Fig. 9.3 Explanation of how HAT medium works



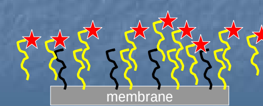
Myeloma cells are HRGPT- and will die on HAT media having hypoxanthine, aminopterin (an antifolate), and thymidine.
Spleen cells are HRGPT+ , so spleen-myeloma (hybridoma) cells can grow on HAT.
(Note: spleen cells by themselves cannot grow in culture.)

Fig. 9.5 Targets for diagnostic monoclonal antibodies

- Polypeptide hormones (chorionic gonadotropin, growth hormone)
- Tumor markers (Prostate-specific antigen)
- Cytokines (interleukins 1-8)
- Drug monitoring (cyclosporin)
- Miscellaneous targets (Vitamin B_{12})
- Infectious diseases (Chlamydia, Herpes, Rubella, Hepatitis B, Legionella, HIV)

Fig. 9.6 DNA diagnostic systems

1. Bind ssDNA (target) to membrane
2. Hybridize to labeled ssDNA or RNA (probe)
3. Wash membrane to remove unbound probe
4. Detect hybrid sequences formed between the probe and target DNA (concern: false +s & -s)



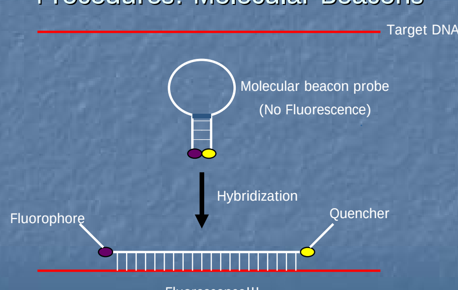
DNA based diagnosis of Malaria and *Typanosoma cruzi*

- A DNA probe from a highly repeated DNA sequence of *Plasmodium falciparum*, the parasite that causes malaria, is used to screen blood samples via hybridization assays
- DNA primers are made against the ends of a 188 bp repeated sequence contained in the protozoan parasite *Typanosoma cruzi*, the causative agent of Chagas disease and used in a PCR/polyacrylamide gel electrophoresis detection method
- Other examples of DNA-based detection: *Salmonella typhi* (food poisoning), certain *E. coli* (gastroenteritis), *Mycobacterium tuberculosis* (tuberculosis), etc.

Nonradioactive Hybridization Procedures

- Use of biotin-labeled nucleotides in DNA probes instead of ^{32}P, then add avidin (streptavidin) which binds to biotin, and then add biotin attached to an enzyme like alkaline phosphase for detection (see Fig. 9.7)
- Note that fluorescent dyes can also be attached to DNA primers for detecting amplified DNA products (see Fig. 9.8)

Fig. 9.9 Nonradioactive Hybridization Procedures: Molecular Beacons



DNA fingerprinting

- Focuses on unique repeated elements, such as VNTRs and STRs, in the human genome (unique in terms of the number of repeats)
- Detection by Southern blotting
- Detection by PCR and gel (or capillary) electrophoresis

DNA fingerprinting

- You're 99.9% identical
- But of course, you are unique--in a genome of three billion letters, even a 0.1 % difference translates into three million differences.
- These differences (or polymorphisms) reside in several places in the genome, often in microsatellites
- Examples of such polymorphisms include RFLPs, VNTRs, STRs, and SNPs

DNA fingerprinting: an example

- D1S80, a VNTR is located on human chromosome 1 and has a 16 bp repeat unit
- The number of repeats varies from one individual to the next, and is known to range from 14-41

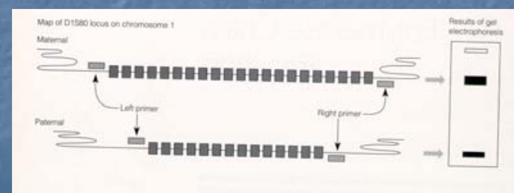
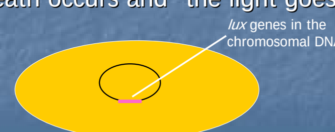


Fig. 9.14 Random Amplified Polymorphic DNA (RAPD)

- Use of arbitrary oligonucleotide primers, usually 9-10 nucleotides long, in a PCR of total DNA to distinguish plant cultivars, animal varieties, and microbe isolates
- A PCR product will be produced whenever two of the oligonucleotide primers face one another and are 100-3,000 bp apart

Fig. 9.16 Bacterial biosensors

- One example involves using *Pseudomonas fluorescens* (genetically engineered for bioluminescence) to monitor pollutants
- If pollutants are present in a sample, then cell death occurs and “the light goes out”



Molecular Diagnosis of Genetic Disease

- Cystic fibrosis
- Sickle-cell anemia