Lecture 16

I. Unknown identification
  A. There are many reasons why one might want to identify an organism.
    1. _____
    2. Development of therapeutic strategies
    3. Epidemiological investigations
    3. _____
    4. Determining players in nutrient cycles
    5. _____
    6. Aiding in restoration efforts
    7. Discover of novel genes
II. Methods for identification
  A. _____
    1. These types of methods are considered the _____ to bacterial identification. We have already covered several of these techniques in lab and you will be using these methods to identify bacterial unknowns in the next several labs.
    2. These methods are based on phenotypic properties of the bacteria. Often they are based on characteristics we can observe in the laboratory. They include:
      i. _____
      ii. _____
      iii. _____
    3. Advantages:
      i. _____
      ii. Several well established protocols exist for common organisms
      iii. Have good _____
    4. Disadvantages
      i. _____
      ii. _____
      iii. Often cannot detect differences subspecies or genotypes
  B. Molecular techniques
    1. _____
      i. ELISA (enzyme-linked immunosorbent assay):
        a. Takes advantage of _____. An antigen is bound to a surface (typically a 96-well plate).
        b. A specially design antibody with an enzyme attached is then added to the plate. Because of the _____of antibodies only the desired antigen will bind the antibody.
        c. The enzyme can then be used to detect binding by reacting with a substrate to produce a _____.
      ii. _____:
        a. Detect specific antibodies present.
      iii. Advantages
        a. _____
        b. Easy to use
        c. Inexpensive
      iv. Disadvantages:
        a. Can have false positives/negatives
        b. _____
        c. Has detection limits so low number of any organism are missed

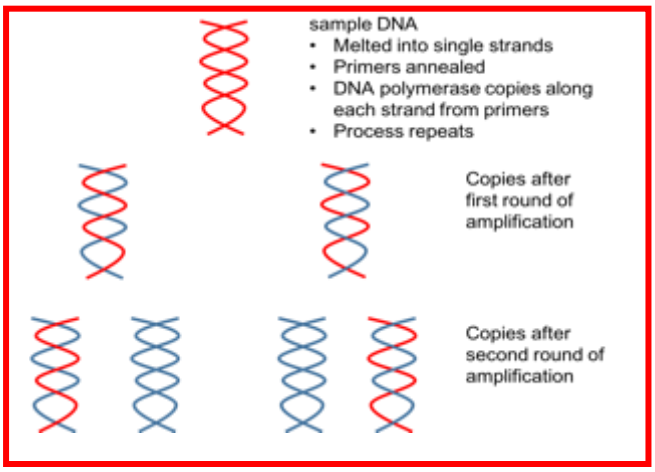
2. Nucleic acid based

i. Amplification techniques: _____ _____

a. Developed in 1983 by Kary B. Mullis, PCR is used for many molecular and microbiology applications.

b. PCR utilizes primers designed to bind to target DNA that has been melted into _____ _____.

c. A _____ then adds nucleotides along the template DNA creating a new double strand of the original DNA sample.



ii. Microarrays:

a. These are similar to immunological assays but use the _____ rather than antigen-antibody interactions.

b. DNA _____can be fixed to a microscope slide at set position and sample DNA will bind to matching probes.

c. A fluorescent signal is then used to detect bind at each site.

d. This allows for 50,000+ unique probes that can detect different bacterial species.

iii. Hybridization methods:

a. These involve the use of a _____ (small sequence of nucleotides) that can bind to specific regions of DNA.

b. Binding of the probe to a target sample of DNA (or RNA) can be detected. There are several techniques that use this hybridization method:

-Northern Blot

-Southern Blot

-*in situ*

- Sandwich

v. Advantages:

a. No waiting for organisms to grow on test media

b. _____

c. Often have better specificity than phenotypic methods

vi. Disadvantages:

a. _____

b. Can be expensive depending on materials needed (fluorescent labels, reagents, equipment)

3. Sequencing based

i. These methods involve sequencing _____ of the DNA. In the case of bacteria the region is often variable regions 3 and 4 of the 16S ribosomal RNA gene.

ii. This gene is used to distinguish differences in "species" on a genomic level.

iii. Sequences of this gene can be compared to _____of gene sequences. You will have the opportunity to try out the NCBI BLAST search tool at the end of the unknowns experiments.

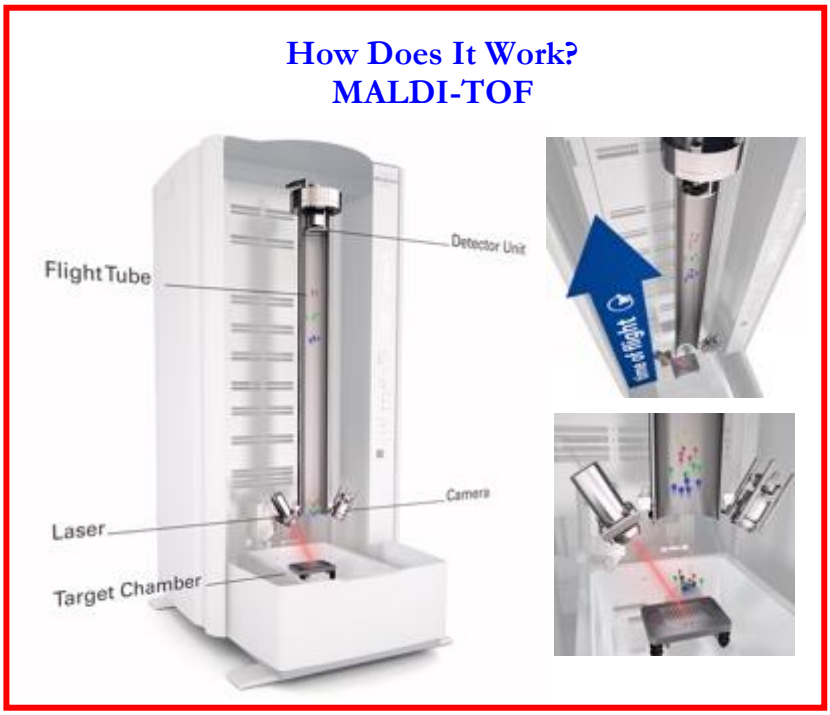
a. Advantages

- High-throughput of many organisms
- Most detailed information
- _____

b. Disadvantages;

- _____
- Often requires training in analysis of sequence data
- Struggles to detect low abundance organisms

4. Other Methods
   i. _____ _____: These methods are based on mass spectrometry (MS) and NMR. Each organism has a unique composition of fatty acids or metabolites that can be detected and used for identification
   ii. _____ (Matrix Assisted Laser Desorption Ionization-Time Of Flight):
      a. This method uses a _____ _____ of selected colonies to ID the organism
      b. Based on the time of flight of ionized samples. Each protein as a different time of flight that can be detected.
      c. Can do several colonies at once
      d. Can ID distinct genotypes.



   iii. Pulsed-field Gel Electrophoresis (PFGE)
      a. This method lyses cells to release the DNA
      b. The DNA is then run on an _____ the sorts the DNA fragments by _____.
      c. The DNA is colored with a dye that can been viewed under UV light.
      d. These size profiles can then be compared to other known size profiles.

III. Identifying your own unknown organisms
   A. _____
      1. Collect a sample from the environment of interest (this will vary depending on the researcher's interest. It may come from soil, spoiled food, or it may be a patient sample: urine, feces, sputum, throat or skin swab, blood, etc.)
      2. Obtain the organism in a _____ using selective and differential media.
   B. Identify the organism
      1. _____
      2. Determine the organism's unique _____:
         i. Nutrient utilization
         ii. Resistance to inhibitory substances (i.e. salts, antibiotics, etc.)
         iii. Enzyme production (catalase, coagulase, hemolysins, oxidase, etc.)
         iv. Motility
         vi. Fermentation end products
         vii. Growth properties (temperature, O_2 concentration/utilization, CO_2,etc.)

IV. Unknowns Procedure
   A. Take one unknown Gram-negative and one Gram-positive. Both the Gram-negative and the Gram-positive should have the same number.
   B. Be certain to correctly label all tubes/plates/slides so as not to confuse the Gram-negative and Gram-positive.
   C. Perform a Gram stain of both unknowns and streak for isolation. It is very important to get an isolated colony!
   D. Be certain that you also receive a clue for both the Gram-negative and Gram-positive. These clues will assist you in writing your hypotheses, which are due (along with your references) _____.
   Please see the description of this assignment on page 161 in the appendix.