Lab 8

Plasmid DNA minipreps

- Minipreps isolate plasmid DNA from small scale liquid cultures; sometimes the colonies formed may not contain the plasmid DNA (false clones) but might be resistant to ampicillin even though they do not contain the plasmid.
- Minipreps help distinguish the false clones from the real ones

How to do a miniprep:

- First centrifuge a small volume of a liquid culture in a neutral buffer w/ RNase (P1)
- Then add P2 which contains sodium dodecyl sulfate (SDS) and sodium hydroxide. This step releases the DNA from the bacteria
- Add a third reagent potassium acetate (N3) which neutralizes the lysis reaction
- Centrifuge again
- Apply the solution to a column that binds specifically to DNA
- Wash the column to get rid of the proteins, salts, and buffers
- At the end, you end up with 50 microL of JUST plasmid DNA. No genomic DNA or proteins!

Lab 9

Quantifying Nucleic Acids

- The amount of nucleic acids is determined using a spectrophotometer that emits UV light
- RNA and DNA absorb UV light; nucleotides absorb around 260nm
- 260/280 ratio is for pure DNA; anything 1.8 or greater is pure DNA or RNA
- How to find your concentration:
    - (Your O.D. X 50ng/micorL) / 1 O.D. = your concentration
- Note: If your sample is diluted, you must multiply your sample by the volume you added!

Restriction Enzyme Digestion of DNA

- Restriction enzymes: exonucleases that catalyze the cleavage of the phosphate bonds w/in both strands of DNA
- Restriction enzymes only cut at recognition sites (specific base sequences)
- These enzymes protect bacteria from invading viruses
- They ONLY cleave dsDNA and leave “sticky ends”; ends that are complimentary to each other; leaves complimentary overhangs
- EcoR1 and Hind3 are restriction enzymes used in this lab

Restriction Mapping

- Restriction mapping is a way to identify DNA fragments based on the principle that any given DNA sequence has a unique pattern of restriction endonuclease sites
- We used this to make sure that the plasmid DNA isolated contained an insert of the PCR gene fragment