

Bioc 463A Molecular Biology Homework Questions

Please feel free to use any references (texts, catalogues, the internet, lab colleagues) to answer these questions.

- 1) Endonucleases recognize specific DNA sequences. Almost all of these recognition sequences are palindromic. Why would it make sense for endonuclease cleavage sites to be palindromic?
- 2) Using the data you collected in class from your restriction digests construct a diagram of the original plasmid. Show the location of the cut sites and number of base pairs between them.
- 3) What is the role of CaCl_2 in the activity of competent cells/transformation? (Hint: wikipedia cell transformation or competent cells)
- 4) Why is buffer added to the restriction digests? Why does EDTA inhibit most endonuclease reactions?
- 5) What is IPTG and what is the molecular mechanism by which it induces protein expression?
- 6) List two primary differences between a cloning cell strain, such as NEB-10s, and an expression cell strain, such as a NEB T 7 *lysY*. Also explain why each difference is beneficial to the researcher.
- 7) Why is kanamycin added to the growth media of cells and how does it function at a molecular level? Kanamycin resistance is conferred on cells by the expression of an aminophosphotransferase. In general terms, how do you think this enzyme renders the antibiotic ineffective?
- 8) Generally, when picking colonies from an agar plate for growing liquid cultures, it is preferable to choose a small single colony as opposed to a larger colony. Why?
- 9) Both plasmid DNA and chromosomal DNA are made of the exact same molecules, NTPs. Why is a miniprep or an alkaline lysis procedure able to separate out plasmid DNA from chromosomal DNA? Why can't you vortex a miniprep solution after the cells have been lysed?
- 10) Explain the role of ethidium bromide (EtBr) in the visualization of DNA on an agarose gel. Be sure to discuss how EtBr labels the DNA and what property allows it to do so.

We hope these questions will enrich the educational experience of this lab! Enjoy!