

Plasmid isolation and digestion, Complementation II, and GMB I Pre-lab. **Due at the start of lab.**

Name \_\_\_\_\_ GSI & Sect # \_\_\_\_\_ Station # \_\_\_\_\_

To answer these questions read all of the lab material on constructing recombinant DNA (pages 64-90) even though you are only doing pages 77-84 for lab 5. You also need to read about complementation (pages 111-118) even though you are only doing pages 111-113. You also need to read about GMB I (pages 135-144). Write legibly. You will NOT be admitted to lab unless you turn in this completed pre-lab at the start!

1) Define the following terms:

binding affinity:

crossing over:

homologous chromosomes:

complementation:

restriction enzyme:

2) Can yeast, deficient in histidine biosynthesis, grow on YPD media? on SD media?

3) Where do you dispose of the used pipette tips and used centrifuge tubes?

4) Discuss the DNA binding affinity of the spin column and how its affinity changes with changes in ionic concentration.

5) During the sequencing reactions either single stranded DNA or double stranded DNA is used as a template (if double stranded DNA is used it must be denatured to yield single stranded DNA). Primers are then added which are complementary to one of the strands. The primers anneal and provide a free 3' OH group to which nucleotides can add. Nucleotides base pair with the template strand and are linked in a 5' to 3' direction by DNA polymerase.

a) In a typical reaction 0.5  $\mu\text{g}$  of DNA is used for the sequencing reaction. If the DNA is single stranded and is 4,000 nucleotides long, how many molecules of DNA is present (in the 0.5  $\mu\text{g}$  of DNA)? The average molecular weight of a nucleotide is 300 grams/mole. Show your work and include all units (include cancellation of units). Give the **exact** number.

b) Four reactions are carried out, each with a **ratio** of 300 molecules of dNTP's to 1 molecule of ddNTP. What is the probability that termination will occur for a given position in the growing strand when it is opposite a T in the template strand? In other words, what percentage of the time will a ddATP be incorporated versus a dATP in the growing strand?

**Pre-lab continued on the other side.**

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6) Double stranded DNA will convert to single stranded DNA if heated high enough; this temperature is known as the "DNA melting point". Which DNA sequence would you expect to have a higher DNA melting point, one with 60% GC or one with 45% GC? Explain your answer based upon the number of hydrogen bonds a GC base pair can form (versus a AT base pair).

7) Diagram the respective stages of the mitotic or meiotic cell cycle of a cell that has a diploid (2N) number of **six** chromosomes. Use different colored pens or pencils. Indicate some crossing over events. It is CRITICAL YOU UNDERSTAND MEIOSIS in order to understand genetics.

MITOSIS

MEIOSIS

prophase

prophase I

metaphase

metaphase I

anaphase

anaphase I

anaphase II