

NAME _____

MICR/MBMB 460: Exam III, Fall 2006

1. **(6 pts)** You are working with a *Bacillus subtilis* PBSX lysogen. You have isolated a mutant that can never be induced and have located the site of the mutation: the *xre* ORF. Describe a mutation of the *xre* gene that could render PBSX non-inducible AND explain the consequences of this mutation (in a few words).

2. **(4 pts)** NAME the three different models of recombination discussed in class and LIST at least two similarities between them.

3. **(4 pts)** A transposon that can only undergo replicative transposition is located in a plasmid. The transposon is now mutated such that the resolvase gene is not functional. DRAW the results of this mutation if this transposon recognized a target sequence in a different plasmid in the same cell. Draw ONLY the final product of this event, NOT the entire process, and label the transposon, the donor and the recipient DNAs.

4. (6 pts) What would most likely be the consequences of a mutation in *clpP* that rendered it non-functional:

a) to a plasmid that contained a PSK (post-segregational killing) module like *phd-doc*?

b) to an *E. coli* cell that contained the *mazE-mazF* operon on its chromosome?

5. (4 pts) How would the effect of the lambda protein RexB on a WILD-TYPE ClpP protein be similar to or different from your answer to 4a (above)?

6. (4 pts) Explain how you might use antisense RNA technology to induce a PBSX lysogen. (2 sentences maximum)

7. (6 pts) DRAW the process that led to the origin of a mobile gene cassette (NOT an integron).

8. (4 pts) Under certain conditions, ONLY short mRNAs are produced from transcription of the Trp operon in the ABSENCE of tryptophan. In 2-3 sentences, explain why this is true.

9. (12 pts) For each of the following merodiploids, indicate whether *lacZ* and *lacY* expression is I (inducible), C (constitutive) or A (absent). (Inducible implies that you would get expression only in the presence of lactose and the absence of glucose.)

A) $lacI^S lacO^C lacZ^- lacY^+ / lacI^- lacO^+ lacZ^+ lacY^-$

lacZ: _____ *lacY*: _____

B) $lacI^S lacO^+ lacP^- lacZ^- lacY^+ / lacI^- lacO^C lacP^+ lacZ^+ lacY^-$

lacZ: _____ *lacY*: _____

C) $lacI^- lacO^C lacZ^- lacY^+ / lacI^- lacO^+ lacZ^+ lacY^-$

lacZ: _____ *lacY*: _____

10. (4 pts) In the partial diploid in B (question #9 above), is the $lacI^S$ considered cis-dominant or trans-dominant? Why?

11. (4 pts) In a completely functional Lac operon, DRAW why β -galactosidase is not produced when the cell is grown in lactose + glucose.

12. (8 pts) State whether the following processes typically involve reciprocal (R) or non-reciprocal recombination (NR).

Recombination between:

_____ the IRS sites in a transposable element to resolve a cointegrate.

_____ the bacterial chromosome and an F' plasmid.

_____ the bacterial chromosome and a small linear piece of DNA obtained through conjugation.

_____ the bacterial chromosome and a linear piece of DNA obtained through transformation.

13. (4 pts) A transposon that can only undergo conservative transposition is located in a bacterial chromosome. The transposon is now mutated such that the transposase promoter is now functional even if the DNA is fully methylated. In 5 words or less, what is the probable result of this mutation?

14. (2 pts) What would be the result if a mobile gene cassette was inserted into an integron such that the 59-base element was adjacent to the 5'-CS? (6 words or less)

15. (4 pts) In one sentence each, explain the importance of transposable elements in:

a) bacterial evolution.

b) human health.

16. (4 pts) DRAW a chi structure (Holliday junction) and NAME the two possible outcomes of resolution of this structure.

17. **(10 pts)** In a brief essay, explain the three different transcriptional regulatory mechanisms that must take place in order for a PBSX lysogen to produce phage particles. (Hint: The emphasis here is on three DIFFERENT regulatory mechanisms that play a role in PBSX transcription.) The three events must be described IN THE PROPER ORDER. (Maximum of 6 sentences.)

18. **(10 pts)** You are interested in enhancing (increasing) programmed cell death when bacteria are exposed to spectinomycin (an antibiotic that affects translation). You decide that the most effective way to do this is by antisense RNA such that the bacteria will be killed only when exposed to the antibiotic. In a brief essay, explain how you could use ppGpp to control the expression of an antisense RNA gene such that an increase in ppGpp production would lead to an increase in programmed cell death. In your answer, you must also briefly explain the normal process by which ppGpp is produced. (Maximum of 6 sentences.)