QUIZ #2

YOUR NAME (please print legibly): _____**ANSWER KEY**_____

As succinctly, but as thoroughly and as accurately as you can, answer the following questions:

(1) When two different temperature-sensitive mutations are present in the same haploid cell background, and the resulting double mutant displays a much more severe phenotype, even at permissive temperature, than either single mutant does by itself, this kind of behavior is an example of (CIRCLE the most correct response): [5 points]

(a) heterozygosity (b) missegregation

(c) synthetic lethality (d) non-allelic non-complementation

(e) dominant-negative (f) none of the above

(2) What is the difference between a shuttle vector and a plasmid shuffle? [10 points]

A shuttle vector is a vehicle for gene cloning that is bifunctional, in the sense that it can both replicate and its presence can be positively selected in two different organisms / hosts. For example, in this course, we have used a shuttle vector that can propagate (ColE1 ori) and be selected (Amp^R) in *Escherichia coli* and can propagate (2 μm DNA ori) and be selected (e.g. *URA3*) in *Saccharomyces cerevisiae*.
The plasmid shuffle procedure is a method used to perform genetic complementation tests, typically for otherwise essential genes, by swapping or exchanging two different plasmids in the same cell. In this approach, a multiply-marked strain (e.g. *ura3 trp1 leu2 his3*) also bearing a chromosomal null (deletion) mutation in the gene of interest (*gene x)* is maintained by a copy of the corresponding wild-type gene (*GENE X*) on a plasmid that carries a marker for which there are independent positive selections for both the presence and absence of the plasmid. Typically, *URA3* is the marker of choice for this application because *ura3* cells that receive a *URA3*-marked plasmid can be selected for on medium lacking Ura; and, conversely, derivatives of those Ura+ cells that have lost the *URA3*-marked plasmid can be selected for on medium containing 5-fluoro-orotic acid (5-FOA) due to the fact that *URA3*+ cells are killed on this medium because the reaction catalyzed by the Ura3 enzyme converts 5-FOA to 5-F-UMP, which eventually gets converted to 5-F-dUMP, a potent inhibitor of thymidylate synthase (the enzyme necessary to make dTMP for DNA replication). The shuffle is performed by transforming the Ura3+ cells with another plasmid carrying the version of the gene to be tested (GENE X*) and another marker (e.g. *LEU2*) by plating the (*leu2*) cells on medium lacking Leu. The resulting Leu+ Ura+ transformants then are plated on -Leu +5-FOA plates. If *GENE X** is functional, then cells can lose the *URA3*-marked plasmid carrying *GENE X*, and hence Leu+ colonies will be observed on the -Leu +5-FOA plates. If *gene x** is non-functional, then no Leu+ Ura- cells will be obtained on the -Leu +5-FOA plates because they will be inviable (since they have no chromosomal *GENE X*, no *URA3*-marked plasmid carrying *GENE X*, and only the non-functional *gene x** on the *LEU2*-marked plasmid is present).

(3) You have been sent a strain with the following genotype: *MAT***a** *ade2 gal4 gal7 gal80 his3 leu2 lys2 trp1 ura3 HIS3::GAL1*$_{prom}$*-lacZ* [pJT110L], where pJT101L is a *CEN* plasmid carrying both the *GAL4* and *URA3* genes. From the list below, CIRCLE all of the media on which this strain will grow (it should be assumed, except where explicitly indicated otherwise, that the carbon source is glucose, and Gal = galactose and Mel = melibiose): [5 points]

YPD YPGal YPMel SC SCGal SCMel SC-His-Ura SC-Trp-Leu SC-Ade

(4) Lysozyme from a certain source has a molecular weight of 10,000 and is available commercially from several companies. You prepare a stock solution of this enzyme by dissolving 2 mg of this lysozyme into a final volume of 1 ml of an appropriate buffer, and store it frozen in a deep (-80 ˚C) freezer. Prior to preparing plasmid DNA from bacterial cells, you thaw the frozen tube of the lysozyme solution and, after gently mixing it, you take a sample and dilute it 100-fold into the bacterial cell suspension. What is the final concentration (in molarity) of lysozyme in the bacterial cell suspension? CIRCLE your final answer, and show your calculations or explain your logic. [5 points]

2 mg / ml diluted 100-fold is 0.02 mg / ml = 20 μg per ml = 20,000 ng / ml.
MW of lysozyme is 10,000 g per mole or 10,000 ng per nanomole.
Therefore, final solution is 2 nmoles / ml or **2 μM**