QUIZ #3

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

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

(1) Based on what you have learned in this course about the conventions for naming *Saccharomyces cerevisiae* shuttle vectors, which of the following plasmids has the highest copy number when present in a yeast cell. CIRCLE the most correct answer. [5 points]

(a) YIp2008 (b) YRp2008 (c) YCp2008 (d) YEp2008 (e) YLp2008

(2) What is the difference between a selection and a counter-selection? Give examples. [10 points]

Positive selection occurs when the particular growth medium (combination or lack of nutrients) and/or conditions (temperature, presence of absence of an antibiotic, etc.) allows for the preferential growth of only those individual organisms that have (or have acquired) the desired genetic constitution. For example, only those ura3 yeast mutants that have been transformed with a URA3-containing plasmid will be able to yield a viable colony on -Ura plates.

A positive counter-selection occurs when the medium and/or conditions are deleterious to the growth of individual organisms that possess a given genetic trait and, thus, only permits individual organisms that lack or have lost that trait to survive and propagate. For example, only those Ura+ transformants that lose the URA3-containing plasmid, and thus have become ura3 again, will be able to survive and yield a viable colony on 5-FOA medium (which also contains some Ura).

(3) Which of the following eukaryotic translation initiation factors is the one that delivers Met-tRNA^{Met} to the initiation complex? CIRCLE the most correct answer. [5 points]

(a) eIF2 (b) eIF3 (c) eIF4A (d) eIF4B (e) eIF4E

(4) The mRNAs encoded by many pathogenic viruses have specialized RNA structures called IRES elements (for "internal ribosome entry sites") that are able to recruit 40S subunits independent of the normal cellular mechanism. In many instances, such viruses further enhance their ability to usurp the cellular translation machinery by expressing a protease that cleaves off the N-terminal third or so of eIF4G. Why will these strategies cause translation of viral mRNAs to be strongly favored over cellular mRNAs in virally-infected cells? [5 points]

Since eIF4G is the scaffold protein that associates with both eIF4E (cap-binding protein) and PAB (poly(A)-binding protein) and mediates their recruitment., and the recruitment of other proteins essential for translation initiation (e.g. the eIF4A RNA helicase), to every cellular mRNA, it is essential for efficient cap- and poly(A)-dependent translational initiation. If eIF4G is broken, even into just two pieces, it cannot serve its function to link the 5'- and 3'-ends of an mRNA; and, therefore, recognition of an intact transcript and stabilization of the cap recognition complex will be greatly impaired. As a result, cellular mRNAs, which depend on their 7-methyl-guanine-containing cap at the 5'-end and their poly(A) tail at the 3'-end for efficient 40S subunit recruitment, will be translated much less efficiently. The viral RNAs that use the special IRES RNA structure to recruit 40S subunits to the viral mRNAs will therefore be translated preferentially by the cellular ribosomes.