2023 AP Daily: Practice Sessions

AP Biology

Session 2 – FRQ (Question 1: Interpreting and Evaluating Experimental Results

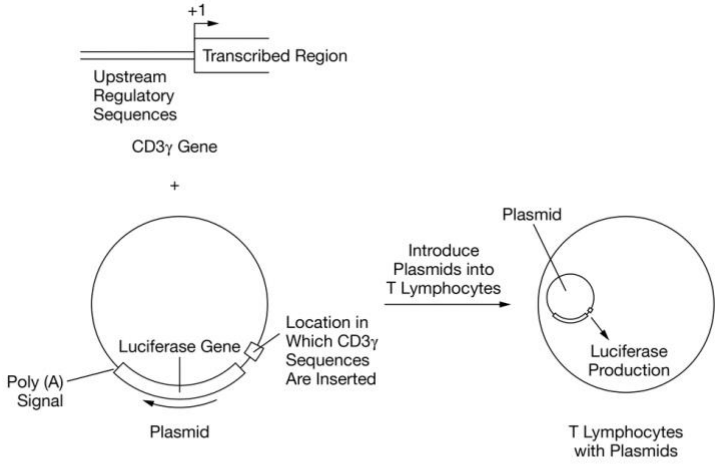

Figure 1. Summary of experimental procedure. A series of plasmids containing fragments of the $CD3\gamma$ upstream regulatory sequences cloned immediately before the luciferase gene were constructed. Each type of plasmid was introduced into T lymphocytes. The amount of luciferase produced by the lymphocytes was dependent on the regulatory sequences present in each plasmid.

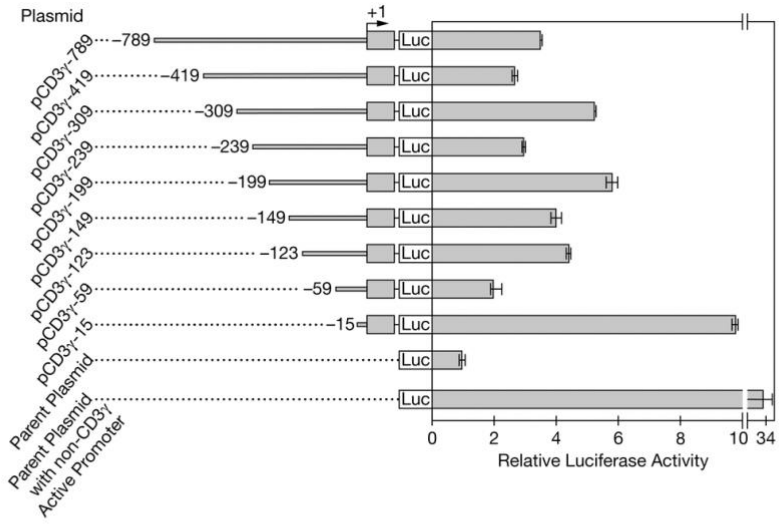

Figure 2. Analysis of the luciferase activity of T lymphocytes containing plasmids with different amounts of the $CD3\gamma$ regulatory sequence. Names of the plasmids are shown on the left. Included regulatory sequences and the resulting luciferase activity are shown on the right. Error bars represent $\pm 2\mathrm{SE}_{\bar{x}}$. The transcription start site is indicated by $+1$.

Source: Unit 6 Progress Check; Taken from: AP Classroom

1. CD3 is a signaling protein that is typically found only in the plasma membrane of immune system T lymphocytes. CD3 is composed of several different polypeptides, including a gamma chain, CD3*γ*. Scientists analyzed the promoter of the CD3*γ* chain gene for regulatory sequences that might have positive or negative effects on expression of the gene.

   The scientists cloned fragments of the CD3*γ* gene that included the first transcribed nucleotides plus up to 789 nucleotides of upstream regulatory sequences into plasmids in which the gene for the firefly enzyme luciferase immediately follows the fragments. The plasmids were then introduced into a line of T lymphocytes (Figure 1), and the cells were allowed to grow for a short while. Because the regulatory sequences of the CD3*γ* gene immediately precede the luciferase gene in the plasmids, the activity, either positive or negative, of the regulatory sequences affected the amount of luciferase gene expression by the T lymphocytes. Luciferase catalyzes a reaction that results in the release of light and is responsible for the bioluminescence (light flashes) of fireflies. By quantifying the bioluminescence, or luciferase activity, in the cells, the scientists were able to determine the effects of each CD3*γ* gene fragment cloned into the plasmids (Figure 2) on expression of the gene.

   a. Identify both the cellular component and the location of the component that is responsible for producing the luciferase protein from mRNAs transcribed in the plasmid-containing T lymphocytes. Explain what dictates to the lymphocytes the correct order in which amino acids should be linked to form the luciferase protein.

   b. Identify the independent variable in the experiment described. Identify the plasmid that was used as a negative control for luciferase activity. Justify including the plasmid with the non-CD3γ active promoter in the experiments.

   c. Identify the plasmid that must contain the CD3*γ* core promoter sequence but the fewest or no negative regulatory sequences. Based on the data in Figure 2, describe the most likely cause of the variation in luciferase activity among the cells that contain plasmids pCD3*γ*-419, pCD3*γ*-309, pCD3*γ*-239, and pCD3*γ*-199. Calculate the approximate percent increase in luciferase activity between cells containing plasmid pCD3*γ*-59 and cells containing plasmid pCD3*γ*-149. Round to the nearest whole number.

   d. Predict the most likely observed level of luciferase activity if plasmid pCDγ3-789 is introduced into nonlymphoid cells such as a line of kidney tissue cells. Provide reasoning to justify your prediction.

Source: Unit 6 Progress Check; Taken from: AP Classroom

2. Certain pathogens have what is known as a mutator phenotype associated with a high frequency of DNA mutations that enable them to change rapidly. *Cryptococcus neoformans* is a fungus that causes severe infections that are often difficult to treat. Scientists assessed whether strains of C. neoformans possess a mutator phenotype.

   The scientists analyzed eleven wild, environmental strains (E1 through E11) and eleven clinical isolate strains (from patients, C1 through C11) of *C. neoformans*. The scientists inoculated an equal number of cells from each strain into 20 replicate liquid cultures in test tubes. After a period of growth, they removed cells from each culture and plated the cells on petri dishes containing a solid growth medium with a chemical that is typically toxic to the fungi. After an incubation period, the scientists counted the number of colonies (each colony is derived from division of a single cell) that grew in each dish and combined the data from the 20 dishes for each strain. Representative data are shown in Figure 1. The data for all environmental strains resembled the data shown for strains E1, E4, and E10. The data for all clinical strains except strains C3 and C6 resembled the data shown for strain C8.

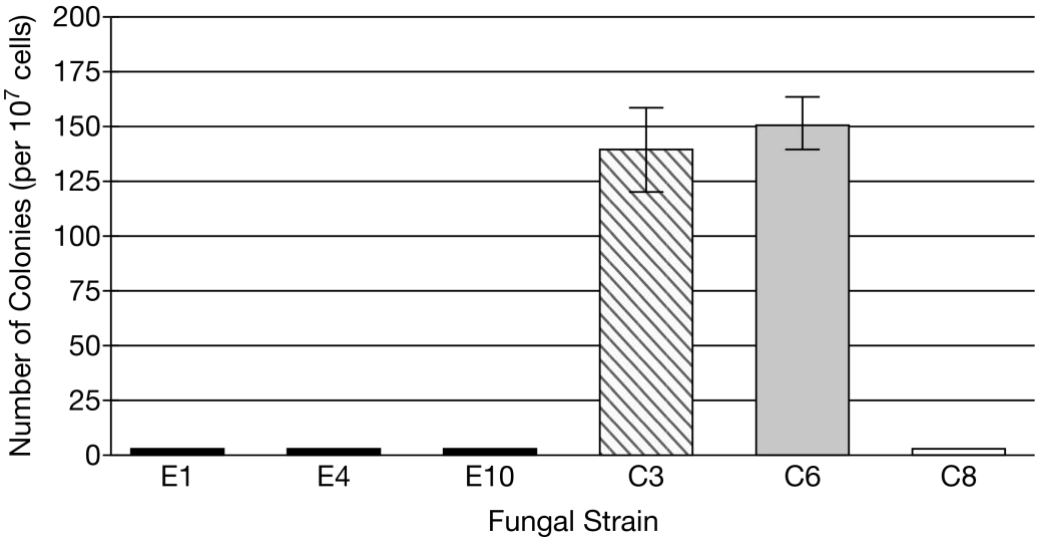

Figure 1. Number of colonies produced from *C. neoformans* cells grown on a medium containing a toxic chemical. Representative data from 11 environmental (E) strains and 11 clinical isolate strains (C). The error bars represent $\pm 2\mathrm{SE}_{\bar{x}}$.

In a further experiment, the scientists investigated the mechanism responsible for the greater number of colonies produced by strains C3 and C6 than by the other strains. Based on DNA sequence analyses of the strains, the scientists introduced a wild-type copy of the fungal *MSH2* gene, a gene encoding a repair enzyme involved in identifying nucleotides in DNA that contain incorrect bases, into cells of strains C3 and C6 and again analyzed the number of colonies that grew in the presence of the toxic chemical (Figure 2).

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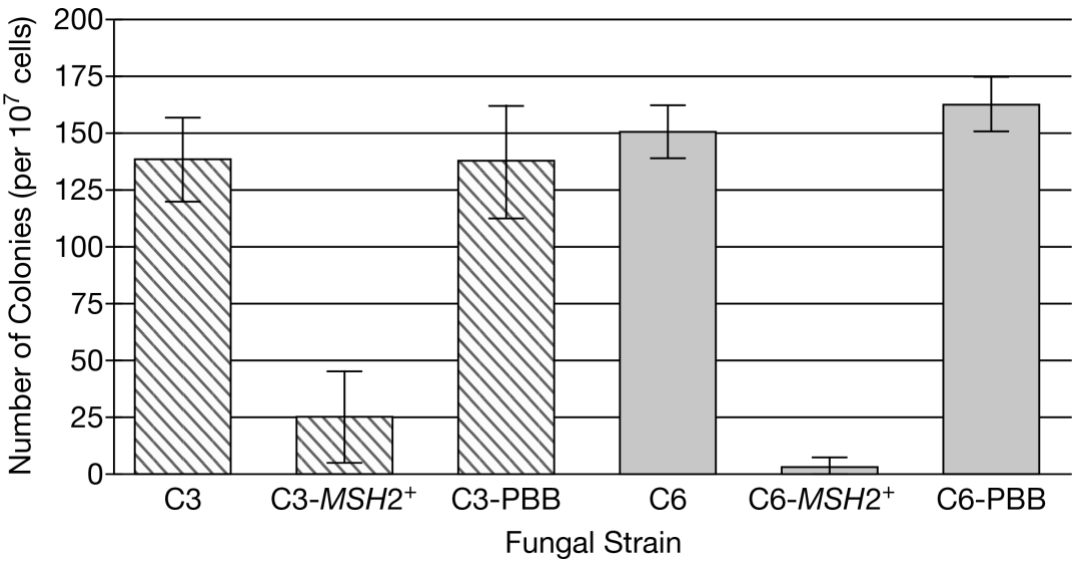

Figure 2. Number of colonies produced from *C. neoformans* strains C3 and C6 cells grown on a medium containing a toxic chemical. A wild-type copy of the fungal MSH2 gene was introduced into strains C3-MSH2+ and C6-MSH2+. The plasmid into which the MSH2 gene was cloned for introduction into the fungi is present in strains C3-PBB and C6-PBB. The error bars represent $\pm 2\mathrm{SE}_{\bar{x}}$.

a. Describe why DNA replication is said to be a semiconservative process. Explain how random mutations such as those in pathogens with a mutator phenotype may arise in the DNA of an organism.

b. Identify a dependent variable in the experiments. Identify the reasoning of the scientists when they tested the number of colonies produced by strains C3-PBB and C6-PBB. The scientists also analyzed the number of colonies produced from each of the environmental and clinical isolate strains when the strains were plated on a growth medium lacking the toxic chemical. Justify this analysis.

c. Based on the data in Figure 1, for each strain describe the relationship between the number of colonies observed and the likely mutation rate of the strain.

d. State the null hypothesis for the experiment whose data are graphed in Figure 2. Provide evidence to support or refute the scientists' claim that more colonies grew in strains C3 and C6 than in the other strains because the genes for proteins that are normally targeted by the toxic chemical contain nucleotides with incorrect bases in the C3 and C6 cells. The scientists additionally determined that the C3 and C6 strains had no decrease in virulence (disease-causing ability) in comparison with the virulence of the other clinical isolate strains and concluded that these two strains have mutator phenotypes. Explain why mutator phenotypes were found only among clinical isolate strains and not among environmental strains.

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