Bio 118 Summer 2003 Homework #2 Name _____

1. Protein-coding genes from one organism may be expressed in other organisms, even in other kingdoms or across the eukaryote-prokaryote divide. This is the basis for the growing, and controversial, practice of commercial genetic engineering.

For example, corn, cotton, other crops plants are now routinely engineered to express the Cry protein. This protein is from the soil bacterium *Bacillus thuringiensis* (Bt). It kills caterpillars, including many of the important crop pests, but is non-toxic to humans. However, first attempts to express Cry protein in plants were unsuccessful. Researchers introduced mutations in the native Cry gene sequence in order to achieve high level of expression. They produced several versions of the Cry gene, each better than preceding version, until they had something that they could take to market.
(Gleave, et al (1998) Molecular Breeding 4:459)

A. For each of the following observations, suggest one type of change in the gene sequence that would give observed change in expression:

Expression Before	Expression After Mutation	Change in DNA?
wild type Cry gene: No Cry RNA transcripts No Cry protein (= version 1.0)	Abundant transcripts (nuclear); low Cry mRNA (cytoplasmic); Cry mRNA too short; very little Cry protein (= version 1.1)	ADD A STRONG EUKARYOTIC PROMOTER. THE BACTERIAL PROMOTER ISN'T USED BY RNA POL II !
same as version 1.1	Abundant Cry mRNA; Cry mRNA too short; very little Cry protein (= version 1.2)	REMOVE "CRYPTIC" SPLICE SITES = SEQ THAT MAY LOOK LIKE SPLICE STES WITH NO CONSEQUENCES FOR THE BACTERIA. IN THE PLANT => PARTIAL SPLICING => NO NUCLEAR EXPORT
same as version 1.2	Abundant Cry mRNA; Cry mRNA full length; small amount of Cry protein (= version 1.3)	REMOVE "CRYPTIC" POLY-A SITES. = SEQ THAT MAY LOOK LIKE "AAUAAA" IN CODING SEQUENCE WITH NO CONSEQUENCES FOR THE BACTERIA. IN PLANT => PREMATURE TERMINATION OF TRANSCRIPTION
same as version 1.3	Abundant mRNA; mRNA full length; large amount of Cry protein (= final product! = Frankenfood v.2)	CHANGE CODON SEQUENCES TO REFLECT PLANT CODON BIAS. BACTERIAL CODON BIAS = VERY SLOW TRANSLATION IN PLANT CELL.

B. One of the changes above involved the introduction of the promoter sequence from the Cauliflower Mosaic Virus (CMV), a common plant virus. Briefly, why did the genetic engineers choose a promoter from a viral gene?

VIRAL PROMOTERS = VERY STRONG, CONSTITUTIVE PROMOTERS. ENGINEERS WANTED TO GET HIGH LEVEL OF EXPRESSION IN ALL CELLS. SINCE CMV IS A PLANT-SPECIFIC VIRUS, THEY EXPECTED THAT IT WOULD BE RECOGNIIZED BY THE GENERAL & CONSTITUTIVE TF'S IN THE PLANT CELL.