Questions on Genetics

a) Why is it easier to ligate sticky ends than blunt ends?

The sticky ends consist of regions of unpaired nucleotides called overhang which readily pairs with another sticky end with a complementary overhang whereas the blunt ends have their nucleotides already paired between the two strands of the DNA hence cannot be easily ligated with other nucleotides (Pedersen et al., 2014).

b) How many bases in a restriction site sequence need to be methylated to protect that site from restriction enzymes?

Only two, that is, Cytosine and Adenine of the four base pairs of the DNA can be methylated therefore enough to repress restriction. The methylation of cytosine is common in both eukaryotes and prokaryotes and differs among species while adenine methylation is common in bacteria, plants, and mammals (Wu et al., 2016).

c) What does a DNA substrate require [or have to have] to be ligated?

A DNA substrate requires four deoxyribonucleotide triphosphates (dNTPs) which are; dATP, dGTP, dTTP as well as dCTP which are cleaved forming deoxynucleotide monophosphates that get inserted into a new DNA strand. Also, the substrate requires ribonucleoside triphosphates (NTPs) which initiate and sustain the synthesis of DNA by synthesizing RNA primers and ATP. The ATP provides energy for the activation of the enzymes at the replication fork.

d) What is the value of using phosphatase when preparing substrates for a ligation?

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