Horizontal Genetic Transfer

1) Bacterial transformation lab - The simplest mode of HGT can be demonstrated in the laboratory. Several protocols and kits are commercially available to demonstrate this process in the lab. In general these types of assays are performed on agar using suspensions of known quantities of bacteria placed onto the agar, with donor DNA being placed on top. After incubation, the “transformed” cells are plated onto selective media (ie media with antibiotics). Calculations such as transformation frequency (number of transformants per CFU of starting cell number), and transformation efficiency (transformants per microgram of donor DNA) can be performed [1].

    a) Carolina has produced a student and instructor friendly kit that can demonstrate transformation, the form of HGT in which free DNA is released from donor cells, taken up by a recipient's cells, and incorporated into the recipient's genome [2]. In this experiment, students use detergent to lyse streptomycin-resistant bacterial cells, which causes them to release the DNA. Streptomycin-sensitive bacterial cells in then incubated in contact with this free DNA which, contains the streptomycin resistance genes. Thus the streptomycin-sensitive cells are transformed and when plated on agar containing streptomycin, they can grow readily. This kit supplies all the materials needed, including sensitive and resistant versions of the bacterial strain, *Acinetobacter calcoaceticus* [2].

    b) An alternative kit is available from Bio-Rad in which students transform bacteria by introducing a gene from the bioluminescent jellyfish *Aequorea victoria* called pGLO [3]. Students have the opportunity to analyze the growth of bacteria on various types of media, and evaluate the roles of external and internal factors on the expression and regulation of genes. In this kit, the students are evaluating the genes that encode the enzymes that metabolize arabinose, a simple sugar (analogous to glucose and lactose in our models). The activity makes use of a promoter region upstream of these genes, which can act as a molecular on/off switch, which can turn on or turn off their expression. When arabinose is present in the environment, the genes are switched on. The kit uses a plasmid that contains the same promoter that switches on and off in the presence of arabinose, only that in this case the genes for the enzymes needed to metabolize the sugar, have been replaced with the jellyfish gene encoding Green Fluorescence Protein or GFP. This means that in the presence of arabinose, the bacteria produce a fluorescent product rather than the enzymes causing the bacteria to fluoresce a brilliant green color that can be readily observed by the students.

2) Conjugation and transduction - Additional kits are available to demonstration of both of these types of horizontal transfer [4-6]

References

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