Bacterial Transformation

Introduction

Genetic transformation is the process whereby a cell incorporates new and foreign genetic material from the environment, leading to a permanent change in its traits. This process occurs naturally in bacteria (using plasmids) and has been adapted by scientists to occur artificially. When scientists introduce genes from other species to form genetically modified organisms, they require a means to control the expression of the introduced genetic material. The plasmids that are utilised will encode a transcription factor that is activated in response to an inducer molecule. This transcription factor will regulate the expression of the introduced gene; hence the target effect will only occur when the cell is presented with the inducer molecule. The most common inducer molecule employed is *arabinose*.

Aim

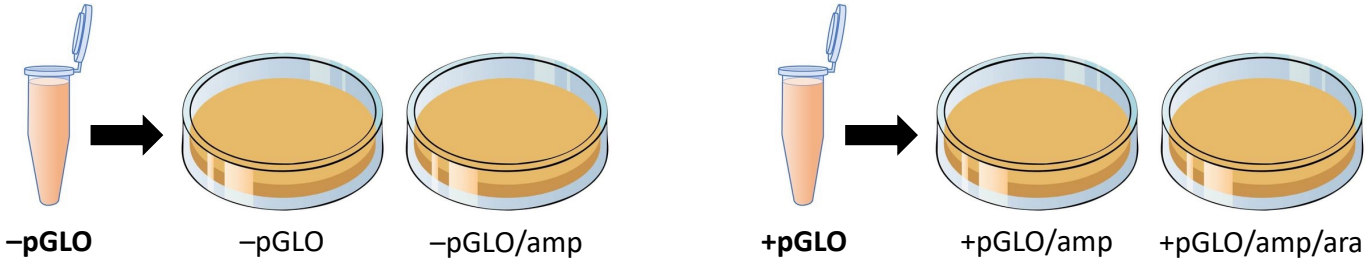
To transform bacterial cells with a glow in the dark protein and use arabinose to control its expression

Materials

Method is based on the pGLO protocol and kit provided by BioRad (https://bio-rad.com/pGLOGenBio)

Method

1. Label one microtube +pGLO and a second tube –pGLO before placing both tubes in the foam rack
2. Transfer 250µl of transformation solution ($CaCl_2$) into each tube and place them on crushed ice
3. Use a sterile loop to pick a single colony of bacteria (non-pathogenic *E. coli*) from the starter plate and transfer the colony to the +pGLO microtube, then repeat this process for the –pGLO microtube
4. Immerse a new sterile loop into the plasmid DNA stock tube and extract a film of plasmid solution
5. Suspend the loop of plasmid DNA into the +pGLO tube only (–pGLO functions as a control group)
6. Incubate both tubes on ice for 10 minutes and use this time to collect four labelled agar plates:
   - –pGLO
   - –pGLO/amp
   - +pGLO/amp
   - +pGLO/amp/ara
7. After the 10 minutes has passed, heat shock the tubes by transferring them to a 42°C water bath for *exactly* 50 seconds before returning to ice for another 2 minutes (the transfers must be rapid)
8. Remove the tubes from the ice and add 250µl of LB nutrient broth using a sterile pipette each time
9. Incubate the microtubes at room temperature (~20°C) for a further 10 minutes before proceeding
10. Using a new sterile pipette each time, transfer 100µl from each tube to the appropriate agar plate
11. Spread the suspensions evenly across the surface of the agar to create a uniform bacterial lawn
12. Seal the plates with tape and place upside down in the incubator for 24 hours to enable growth
13. When colonies have formed, use a UV light to determine if the individual cells are fluorescent



Results

Identify which plate(s) have fluorescent colonies and explain with reference to transcription factors

Discussion

1. Explain the purpose of the two –pGLO plates in the experiment

2. Explain the purpose of the +pGLO/amp plate in the experiment