Group manuscript

Bacterial cells are a common choice for in vivo replication of DNA of interest, and in this study, the heat shock method was employed for bacterial transformation. Plasmids, which are DNA molecules themselves, were used as expression vectors for the DNA of interest, the GAP gene. Because only transformed cells exhibit antibiotic resistance, trans armed cells survived on plates containing inclining. Only those cells that took up plasmid s containing the GAP gene fluoresced in IV light.

By restriction enzyme analysis and gel electro prophesiers, the relationship between genotype and phenotype was observed using isolated p zamias from the bacteria. Because the presence of the GAP gene codes for fluorescence, it is expected t hat a genotype coding for the protein would express the glowing phenotype. Regular action of GAP gene expression was observed in samples that were grown with rabbinate, inimical in, and varying amounts Of glucose. Our results Suggest that the presence Of glucose in the s roundingenvironmentinhibited transcription from the rabbinate bad promoter. Age 3 INTRODUCTION This study examined the transcriptional regulation Of the rabbinate Oberon pr emoted found in Escherichia coli (E. Coli). To facilitate this study, the Green Fluorescence t protein (GAP) was utilized as a reporter gene with the rabbinate promoter. The KEEP gene w as 772 base pairs (BP) long and was extracted from Quarrel Victoria. This gene was implanted into plasmids, which were inserted into the E. Coli through bacterial transformation (3). In order to obtain enough copies of these DNA samples in a reasonable ammo NT of time, two methods can be used.

The first is considered in vitro , or in glass, (such as a test tube) and is known as polymerase chain reaction. This method, in which a machine heats t he

DNA sample ND Tag polymerase clones the DNA, is expensive and less convenient, so it is not always used. The other method is In Vivo , or in life, and is called transformation. This method was used in this experiment by shocking E. Coli bacteria with heat in order for them to take in plasmids that were transformed to contain the GAP gene. Not all bacteria were transformed, and not all transformed bacteria contained the GAP gene.

To differentiate between transformed and n untransformed bacteria, they were grown on inclining, as the transformed bacteria were rest assistant to impact Olin but the untransformed bacteria were killed by it. The plasmids with and with out the GAP gene were differentiated by visualization under IV light (4). The genotype of the remaining plasmids, both transformed and nontransparent med, were then tested to determine the relationship between the genotype, or plasmid c imposition, and phenotype, or presence of fluorescence.

The plasmids were removed from the e bacteria, with some samples left whole as controls and others cut into pieces by restriction enzymes. In this case, doll, originating from Hemophilia influenza , was the enzyme used to cut the plasmids Page 4 at their respective Hind doll sites, where the GAP gene would have been inserted. This was done to determine whether or not the KEEP gene was taken in by the plasmids when it was electrophoresis, as the difference in size of the pieces was observed in the gag arose gel (4).

Even though a transformed bacterium may have had the GAP gene in its insert Ted plasmid, it needed the promoter bad (consisting of genes Arab, area, and award) and t he GAP gene in the right direction and position in

order for the bacterium to have the potent al for fluorescence. It may still not have fluoresced if there was not enough rabbinate present for the bad promoter to run, or if a high concentration of glucose inside the cell was present to rep as the bad promoter.

With all the necessary genetic coding for fluorescence, the amount that was visible depended on the amount of glucose present, as no glucose caused it to floorer see brightly, and a low concentration made it glow dully (4). Each step of this experiment was vital in analyzing the transcriptional regulate on of the rabbinate Oberon promoter. Through bacterial transformation, recombinant DNA and cloning methods were used in order to insert the GAP gene into the plasmid.

The pellet of cells was then resume need using a pipette and vortex mixer. This allows for a XX concentration to be obtained. After spreading, the four plates were incubated upside down (overnight at 370 C) in a microbial incubator. Following incubation each plate was placed upside down n a IV box and photographed. Restriction Analysis In restriction analysis, two restriction enzyme digestion reactions (one uncut b Y enzyme, one cut by enzyme) occurred for each of the two DNA samples (nonresistant and non page 6 fluorescent, resistant and inflorescent, and resistant and fluorescent).

For the uncut samples, components were added in the following order: Pl XIX Buffer (supplied by environment by life technologies"' containing: 100 mm Trisect, pH 7. 5 100 mm Magical mm Theoretical 500 mm Nasal), 1 Pl Water, and 5 Pl DNA For the uncut samples, components were e added in the allowing order: Pl XIX Buffer, Pl Water, pi DNA, and III Handbill NZ. (sup plied

by invitation by life technologies""). After all components were added they were mixed by overexerting and collected at the bottom of the tubes by using the microelectronic gem The tubes were then incubated for 30 minutes at 37 co.

Following incubation, Pl of XIX loading g buffer (1% (w/v) SD (sodium decoded sulfate) 50% (v/v) glycerol 0. 05% (w/ v) bronchiole blue) was added to each tube. The samples were mixed by overexerting and collected at the e bottom of the tubes using the microelectronic- Agrees gel electrophoresis was prepared by ding Pl of 1 KGB plus ladder (supplied by environment by life technologies"") into the first and final well. 12. Pl of each sample was then loaded into remaining wells on the gel and the electro prioress was run for 1 hour at 1 VIVO.

The gels were then placed in a IV box and photographed. Using thephotoof the gel, the genotypes of each sample were verified. This was done by finding the size of fragments through comparison with the DNA standard ladder. Gene Expression TO allow forobservationOf phenotypes gene expression, bacteria were transfer erred to three types of media: inducing, introducing, and repressing. Five plates were used: LB,'Amp, LB/Marry, LB/Amp/AR/Glue 0. 2%, LB/Amp/AR/Glue 0. 5%, LB/ Amp/AR/Glue 2% (xx).

On each plate, bacteria with empty plasmids and therefore no GAP gene for flour essence were spread on one half, and bacteria with plasmids containing the GAP insert were e transferred to the Page 7 other. Both of these types of cells came from a master plate. Using a sterile to toothpick, each type of bacteria was patched in the appropriate area of each plate. GAP+ bacteria

were patched in a "+" shape, while GAP bacteria were patched in a shape. Plates were labeled properly and Leary and were placed Poseidon in a ICC incubator overnight.

Plates were e observed for fluorescent bacteria on the IV transformational box after 24 hours, after 72 h ours, and again after 96 hours. Page 8 RESULTS Bacterial Transformation In order to investigate GAP gene expression, it was first necessary to obtain co pies of the DNA of interest through bacterial transformation, which allowed for plasmid (and sometimes GAP) uptake by numerous bacterium. Phenotypes results from this procedure can suggest possible genotypes. Transformation plates that were prepared after heat shoo KC transformation can be Seen in Figure 2.

Individual colonies were visible on each AMP+ plate, while a lawn of bacteria had formed on the AMP plate and individual colonies were not discern enable. Under IV light, fluorescence was observed in those colonies expressing the GAP gene. If guru E highlights the difference observed between glowing and knowing bacterial colonies. The fraction of colonies that appear fluorescent and are assumed to be GAP+ is noted in Table e 1 along with complete results of this bacterial transformation (4).

Structural Analysis by Restriction Analysis and Gel Electrophoresis TO confirm the structure Of DNA plasmid genotypes, samples were run through h gel electrophoresis after being treated with a restriction enzyme specific for cleave ins the gene of interest (GAP). Standards were run along with each of the components on the electrophoresis gel. In order to determine size in base pairs of fragments of interest, a graph of the e relationship between the

size and migration of the bands in the 1 KGB plus DNA Ladder was assembled (Figure 4), and a line of best fit was determined.

The relationship between the base 1 O log of size and migration is linear, and graphing them together gave a trend line with an ex. action useful in determining the size of experimental fragments with known migration values. These were the fragments obtained by cleaving the plasmids with the Handbill restriction enzyme. Table 2 page 9 organizes the sizes and migration distances for the fragments of the standard included during electrophoresis. These values were used to construct the calibration curve m mentioned before (Figure 4).

Figure 3 shows the agrees gel obtained by gel electrophoresis. Ta able 3 lists all sizes determined based on comparison with the calibration curve generated from migration standards (Figure 4). Sizes are noted for both the vector and the insert (4). Analysis of Reporter Gene Expression Investigation of gene regulation and interaction of environmental rabbinate a ND/or glucose with genotype required GAP+ cells to be spread on various plates, an d fluorescence to be observed over time.

Table 4 summarizes the observations of the phenotype o f patches streaked onto AMP plates containing or lacking rabbinate and/ or glucose. Glowing patches suggest expression of the GAP gene. Observations show that the rabbinate sample pop site for the GAP insert fluoresced brightly as time went on. The sample with rabbinate and 0. 2 % glucose increasingly fluoresced over time, while plates higher in percentage glucose c imposition did not

fluoresce. Cells that were GAP were also spread in order to serve as a surrogate et marker.

These cells do not contain the gene for GAP, so they will not fluoresce under IV light . This gives a comparison, making it easier to determine if cells are expressing GAP fluoresce once or not page 10 DISCUSSION Regulation of Gene Expression: The samples that were grown with rabbinate, inclining, and varying amounts of glucose showed that the presence of glucose in a bacterium's surrounding environment NT can affect the ability of its rabbinate bad promoter. The plate with no glucose added flour cede brightly, while the plates with glucose added showed very little to no fluorescence.

The only plate with glucose added that fluoresced in the end was the plate with the least glucose added These results are due to the glucose inhibiting the rabbinate Oberon from trap inscribing the bad promoter DNA. When glucose is present in a bacterium, the cell metal likes the glucose instead of the rabbinate, and the rabbinate Oberon is not utilized. However, when the cell is lacking glucose, it reaches a state of " hunger" and begins producing cyclic adenosine Mephistopheles (CAMP). This reacts with the CAMP receptor protein (CROP), who chi allows the cell to use rabbinate to induce the transcription of the rabbinate bad prom otter.

This promoter contains the genes Arab area , and award, which are part of the rabbinate Oberon. This system can only function if rabbinate is present in the cell; otherwise the gene arc will prevent the rabbinate Oberon from carrying out transcription by forming a " knot," or loop in the DNA The rabbinate bad promoter reacts with the GAP gene to show when the Arabian

SSE Oberon is in use and how strongly it is induced by rabbinate (5). The plate with the least glucose added began to glow over time, as the cell be an using up the glucose in its environment by metabolize it for energy.

It started to FL recurrences dully once the concentration of glucose was not high enough to fully repress the AR baboons Oberon, showing that there is a range of repression and induction, not just a state of " on" and " off' for page 11 these function. The more the bacteria used the glucose, the less of it was arrow ND to repress the Oberon, which is why its fluorescence strength grew over time. If the study WA s to be continued past the 96 hour mark, all of the plates would have eventually fluoresced as t hey used up their loses resources and began activating the rabbinate Oberon (4).