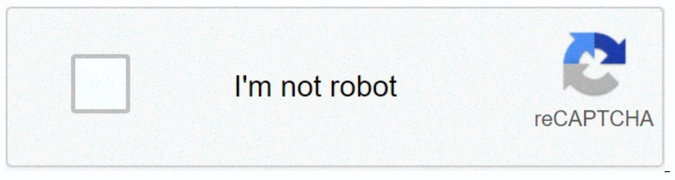

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Pgreen transformation lab answers

The transformation of bacterial cells is a useful experiment to help develop an understanding of transformation by plasmid DNA. This experiment involved four different scenarios of bacterial cells on agar plates. The scenarios were as follows, a plate with plasmid, an outside and a plate with ampicillin and plasmid and one with ampiciline and without plasmid. Transformation efficiency was then determined by analyzing the amount of resulting colonies that were created. Can we help with your assignment? Let's do your homework! Professional writers in all disciplines are available and will meet your task deadline. Free proofreading and copy editing included. Transformation is the introduction of foreign DNA, in this experiment by plasmid, into a bacterial cell. Transformation is essential in molecular biology and the observable results of this experiment are evidence of the effectiveness of transformation. Since the bacteria E. coli are not naturally competent cells, the E. coli bacterial cells and pVIB plasmid should be mixed with calcium chloride. This solution must then undergo a heat shock in water at 42 degrees to create a draft and sweep plasmids into the pores of the bacterial membrane. After preparing two bacterial cells, one with plasmid and one without, another agar plate is produced, where ampiciline is introduced. Ampicillin is an antibiotic that is known to treat bacterial infections, in this experiment ampicillin's role is to kill all bacteria that do not undergo transformation. The purpose of this laboratory was to develop an understanding and appreciate the results of transformation as well as focus on the effects of ampicillin on pVIB plasmid. Materials: 2 sterile 15-mL tubes 4 sterile transfer pipettes 3-4 glass beads 4 sterile transfer columns 2 LB agar plates 2 LB agar plates with ampicillin pVIB plasmid 500 µL caCl2 LB Broth cup of 42 degrees water cup full of ice-waste beaker Observations and results Table 1.0: Results LB-plasmid Prediction: Lawn Cause: bacteria will line Luria Broth observed result: lawn LB + plasmid Prediction: Lawn Cause: same as plate 1 Observed result: Lawn LB/ AMP-plasmid Prediction: no growth Cause: ampicilin kills bacteria Observed Result: no growth LB / AMP + plasmid prediction: isolated colonies Cause: choose for those who took up plasmids observed results: 2 colonies LB + pla Throwaway (positive control) - lawn LB-plasmid (positive control) - lawn LB / Amp + plasmid (experimental) - 2 colonies LB / Amp-plasmid (negative control) - no growth Analysis and conclusions My observed results (listed above) were very accurate with my prediction. As predicted, LB/Amp plasmid showed no growth due to the fact that ampicillin killed bacterial cells and there was no plasmid present for resistance. As expected my group and I two isolated colonies within LB/Amp+plasmid and and LB plasmid and LB+plasmid showed lawn colony growths. Both LB+plasmid and LB plasmid plates exhibited lawn colonies. These results tell us that the experiment was performed effectively because the bacteria were able to line luria broth. Also, because there was no ampicillin present there was a large growth of bacteria because the bacteria were replicating without being destroyed by other factors. LB/Amp plasmid and LB plasmid had results that contrasted with each other. LB/Amp plasmid showed no growth due to the fact that ampicillin killed the bacteria. But LB-plasmid showed a lawn growth because there was no presence of ampicillin. These results clearly show what a major impact the presence of ampicillin in bacterial cells has. LB/Amp+plasmid and LB/Amp plasmid also showed contrasting results. While LB/Amp plasmid showed no signs of growth, LB/Amp+plasmid showed isolated colonies. This is because the bacteria that picked up plasmid were able to withstand ampicillin. This data from the experiment shows us that the process of converting plasmids is very effective. Both LB/Amp+plasmid and LB+plasmid results showed signs of growth, but they differed in scope. LB/Amp+ plasmid showed isolated colonies because only the bacteria that took up plasmid glowed. But LB+ plasmid showed a lawn growth because the bacteria were able to line luria broth effectively. These results show that ampicillin will only have an effect if the experiment is carried out very accurately. In this experiment, we choose for fluorescence that occurs when a bacterium has taken a plasmid. To identify if plasmid worked effectively my group and I was taken to a dark closet we were observing the plate to see if it would fluoresce. Fluorescence was an indication that the bacteria survived and have taken plasmid and therefore because we had two isolated colonies, we conducted the experiment effectively. The phenotype of the colonies is proof that they were transformed effectively. Fluorescence's appearance shows that the bacteria took plasmid. The plate that I will first inspect to conclude that the conversion was done successfully is LB/Amp+ plasmid. This is because for a transformation to be done effectively the foreign DNA must be inserted into the bacterial cell by a plasmid. Of course, one would not look to the plates with-plasmid so the only two left are LB+ plasmid and LB/Amp+ plasmid. LB/Amp+ plasmid would be the better choice because ampicillin will kill all the bacteria that are not taken up by a plasmid. Therefore, the plate will only fluoresce if the transformation was successful. Total mass = volume x concentration 0.05 µg plasmid = 10 µL plasmid x 0.0005 µg/µL 250 µL LB + 250 µL CaCl2 + 10 µL plasmid = 510 µL cell suspension Volume suspension dispersion / total volume suspension = fraction spread 100 µL cell suspension / 510 µL total suspension = 0.196 (fraction dispersion) Total mass plasmid x fraction spread = mass plasmid DNA spread 0.05 µg plasmid x 0. 196 = 0.0098 µg Colonies observed/ plasmid mass spread = transformation efficiency 2 colonies / 0.0098 µg plasmid = 2.041x 102 colonies/µg plasmid I think there are various factors that affect transformation efficiency. What I think is the most important factor is the completion of heat shock. If heat shock were to happen incorrectly then plasmids would be able to get into bacterial cells and therefore transformation would be able to occur. In order for the heat shock to be properly preformed, the temperature must not be too high or too low, as this will affect the rate at which the plasmids enter the pores of the bacteria. Another factor that is important in this experiment is the amount of E. coli and pVIB plasmids that are transferred to the various plates. If the amount transferred is too much or too little, the transformation may not even occur. The last factor that plays a major role in the effectiveness of this experiment is the temperature at which the plates are incubated. The plates should incubate at 30 degrees and if the temperature is even a few degrees from the bacteria will be able to fluoresce. If this were to happen our data would be wrong because we would be able to count the total number of colonies. The transformation of bacterial cells is a useful experiment to help develop an understanding of transformation by plasmid DNA. 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