Biochemistry report assignment

ASSIGN BUSTER

The main goals of this experiment are testing an alternative procedure called “ boiling lists”, evaluating the quality of the purified plasmid for restriction digests, and identifying the mislabel plasmid. The plasmid DNA from a carrier E. Coli strain was purified by the boiling lists. In the boiling lists method, the bacterial cells were given momentary heat treatment in boiling water in presence of loosely and triton X-OHIO.

Since the plasmid DNA is small in size, it comes out from the bacterial cell, while the genomic DNA remains trapped in the cell. Successive high-speed centrifugation separated the plasmid DNA from rest of the cell debris, which form pellet. The pellet was removed and plasmid DNA was recovered by suppression precipitation method. Restriction enzymes are enzymes that selectively cut a DNA molecule at a particular place called restriction sites. Restriction enzymes also recognize a specific sequence of nucleotides, which vary between 4 and 8 nucleotides, in particular, palindrome.

These restriction enzymes were kept in a 50% glycerol buffer that does not freeze at -20 co. The four available enzymes used for this experiment were Score, Avail, Hinkle, and Rasa. The lengths of the DNA fragments from the restriction digests are used to map the relative locations of the four available enzymes in the plasmid. From this restriction sites, the unknown plasmid will be identified. Agrees gel electrophoresis is an easy way to separate DNA fragments by their sizes and visualize them. The length of the fragments from digestion is estimated by comparing the results obtained from the reference DNA reagents.

Materials and Methods Plasmid purification. The bacterial culture used for this experiment is JIMMY strain of E. Coli carrying an unknown plasmid. For 20 seconds, 1. 5 ml of bacterial culture was centrifuge at maximum speed in a microelectronic to pellet the bacteria. Before lysine the bacteria, 500 LO of Dense-free water was used. The bacterial pellet was respond in 350 LO of STET solution (8 % w/v sucrose, 50 mm Tries-HCI (pH 8. 0), 50 run EDIT (PH 8. 0), 5 % w,'V Triton X-100, 700 go,'t-ml loosely, and 250 go,'t-ml Arenas A.).

The 700 Go/ml loosely from chicken egg was used to weaken the bacterial cell- wall pedagogical. Triton X-OHIO and heat disrupt the membrane lipid bilateral. The bacterium was fully lased after it was placed in a boiling water for 1 minute. The proteins, such as Dense, Arenas, loosely and all of the proteins from the lased cell were extracted by adding 3 L to alcohol solution (24: 24: 1), overexerting for 5 seconds, and centrifuging for a minute in a microelectronic at maximum speed. The standard conditions for ethanol precipitation are 0. 3 M Cook with 2. 5-3. Volumes of absolute ethanol. The pellet was completely dried in a Speedway for 10 minutes, and then the pellet was dissolve in 20 LO of ET buffer (10 mm Tries-HCI, pH 8. 0, 0. 1 mm EDIT) for the final suspension. DNA characterization. The plasmid DNA was diluted by adding 2 LO of the plasmid DNA from plasmid purification with 500 LO of ET buffer (10 mm Tries-HCI, pH 8. 0, 0. 1 mm EDIT). The wavelengths used in this experiment are 230, 260, and 280 NM. The spectrophotometer model used in the experiment is Beckman Coulter DIG 730 Life Science Jives Spectrophotometer.

The equation used for calculating DNA incineration is as follows: [DNA] (Go/ml) = Axis 50. Restriction digest methods. In each of the restriction digests, it contained 1 LO of XIX N. B. enzyme buffer (IX is 20 mm Tries-acetate, 50 mm potassium-acetate, 10 mm magnesium-acetate, 1 mm Theoretically pH 7. 9 at 25 co), 1 LO of BAS (10 MGM/ml), 1 LO of restriction enzyme (for double digests, 1 LO of each enzyme was added), and 1 Go of plasmid DNA. Subsequently all the components for the restriction digest were combined and mixed, the tubes were incubated at 37 co in a heating well for approximately 60 minutes.