Mutation in e coli bacterial cells



Mutation in e coli bacterial cells – Paper Example

In this experiment, mutation in E. coli bacterial cells is learned. Mutations occur when DNA sequence are altered: either deletions or insertions of a single or multiple base pair that can lead to frameshift mutations. Mutation frequency measures how frequently the cells are mutated in a given population. Spontaneously occurring mutation is not frequent (10-5 to 10-10 mutation frequency). 2) Therefore, to induce the mutation, it would be necessary to use chemical mutagens. It incorporates into DNA as adenine or guanine leading to mispairing of guanine; AT to GC transition and GC to AT can occur as a consequence of 2AP induced mutation. It is relatively weak than other chemical mutagen and the procedure is simple to mutagenize E. coli with 2AP. Therefore, it is relevant to use 2AP. E. coli was exposed to 2-AP and the effect on E. coli by 2-AP was observed by comparing with E. coli grown without 2-AP. In the second part of the experiment, insertional mutagenesis of Tn10 are used. (1)

E. coli strain used was CC102 (ara â[†](gpt- lac)5 thi rpsL/F'lacZ- Y+ A+ proA+B+).(refer) This strain is auxotrophic: it requires thiamine, is streptomycine resistant and it is missing Lac Z gene. CC102 is missing LacZ, therefore, when the mutation occurs by 2AP, it could gain the LacZ function by mutation. The mutation is done in random locations. The mutation can be tested by selection, the growth condition in which only a specific type of strains can grow. Afterwards, it can be tested by screening with IPTG and Xgal containing plate where it would show blue when Lac Z is active. However, this screening method is not used in this experiment. Instead, replica assays are made to identify auxotrophic mutants. Since auxotrophic mutants will not grow on minimum glucose, the number of auxotrophic mutants can be

Mutation in e coli bacterial cells – Paper Example

Page 3

identified by comparing the colonies in "LB tet" medium containing plate to the colonies in minimum glucose. In order to determine mutation frequency, viable count from the LB plate is used as number of colony formed in a plate and number of colony grown in different media are used to determine the mutation frequency. (1)(2)

Insertional mutagenesis is done by Tn10. Tn10 is a transposon that carries tetracycline resistance gene. For this to be inserted to the cell, lambda phage was used. To prevent the lambda phage from lysogenizing, its attachment site was eliminated and was replaced with Tn10. Then, site specific recombination would not occur. Tn10 would be successfully inserted by lambda phage and create random mutations. Also, the mutated cell will now be resistant to tetracycline. (1)

Material and Methods:

Please Refer to Biol 368 lab manual*

All procedures are performed according to the BIOL 368 lab manual (Concordia Biology Department 2010) except the followings: in part A, step 6, we added 1. 0 ml of Tryptone broth containing sodium citrate to each tube instead of 1. 8 ml. Also, for the section data of part B, given data is used instead of our actual section data.