Gene Expression Lab Report

Name

Institution

**Abstract**

This lab report is intended at documenting laboratory activity regarding gene expression in terms of “survival” and “illumination” of bacteria in controlled and experimental conditions. Bacteria were inoculated with genes of interest through plasmids (one lux plasmid for illumination and one controlled pUC18). Both the plasmids contained Ampicillin resistance gene responsible for bacterial survival through deactivating antimicrobial peptides (Amp); which kills gram positive and gram negative bacteria. After series of experimentation, it was revealed that genes can be used to develop desired characteristic within the host cell as bacterial cell grew even in the presence of antimicrobial peptides when Ampicillin resistance genes were introduced and they demonstrated light reactions when lux containing plasmid was introduced. Transformation efficiency of bacterial cells was also calculated using scientific formula in the end.

**Introduction**

Gene is referred to as DNA or RNA nucleotide sequences responsible for developing structural, functional and biochemical properties of cell through the process of coding (Markus & Mariana, 2006). Genes are composed of DNA, RNA or proteins. Gene expression is the process responsible for utilizing genetic information for synthesizing functional gene product. The end products typically encapsulate proteins with the exceptions of functional RNA in case of non-proteins coding genes e.g., transfer RNA (t-RNA) and nuclear RNA (Chen & Dubnau, 2004).

During the process of gene expression, the first step is known as transcription involving transformation of DNA into RNA. The transcribed RNA is either directly functional or functions are an intermediate template for protein formation that performs specialized functions. The second step involves translation of transcribed RNA molecules into poly-peptic sequences responsible for executing particular cellular functions (Chung, Niemela & Miller, 1989).

Typically, phenotypic traits are inherited when transmission of genes takes place from parent cell to off-springs. Specialized DNA sequences responsible for unique genetic make-up are referred to as genotypes. Environmental and developmental factors coupled with the genotypes determine the nature of phenotype that individual will be more likely to express through his physical, cognitive, emotional and behavioral ramifications (Noble, 2008).

 A bulk of literature is evident that poly-genes and interactional process between genes and outside environment of individual are the powerful determinants of biological traits. Out of these biological traits, some are instantly visible including height, weight, number of limbs, skin and eye color whereas some are latent encompassing risk of acquiring physical or mental disorders, blood group type, and numerous fundamental biological processes that attempt to aggregate life (Benzer, 1959). This process is utilized by all the known life forms including eukaryotes and prokaryotes for generating macromolecular machinery to sustain life.

 As genes are carrying genetic materials known as DNA, it is typically assumed that only cell nucleus contains DNA, which is nothing more than a misconception. Plasmids are small round shaped structures carrying DNA particularly in bacterial cells. DNA present in plasmids is independent in terms of physical placement and functional activities e.g., replication and translational aspects.

 Plasmids are carrying certain genes favorable for organisms’ survival particularly for providing resistance against antibiotics to bacteria. Chromosomes are large enough and contain essential genetic information for surviving under optimal conditions whereas plasmids are comparatively smaller and contain information about how to survive in the drastic environmental conditions (Inoue, Nojima & Okayama, 1999).

 Within the laboratory settings, synthetic plasmids are widely used for inserting desired genes into host body which then undergo replication and translation forming colonies—acting as vectors. The process of transformation allows the introduction of plasmids into host cell. The successful completion of transformation requires three conditions; host for the insertion of foreign DNA, vector for introducing foreign DNA into host cell and identification and selection of transformed cells (Hanahan, Jessee & Bloom, 1991).

In this lab report, the practical manifestation of transformation will be elaborated. Following materials will be used to accomplish transformation; the host cell, gene of interest and vector. The host cell selected to undergo introduction of foreign gene was *E. coli* bacterium. This specie was selected for several reasons; it has one chromosome made up of approximately 5 million base-pairs with rapid growth rates and short reproduction time (Noble, 2008).

Gene of interest was *Ampicillin-resistance gene* which enables *E. coli* to develop immunity against antibiotics. It codes for the enzyme known as *beta-lectamase* that in turn deactivates the ampicillin along with the other antibiotics. The other gene of interest is *lux operon* which facilitates the production of luminescent proteins that in turn generate light reactions when chemical energy in converted into light energy.

Vector mediates the incorporation of foreign DNA into the host cell. The selected vector for laboratory activity was plasmid—pUC18. Plasmids were used because they are small in size, less susceptible to physical damage and replicate more effectively giving away numerous copies of DNA. Calcium chloride makes the bacteria more welcoming for plasmid which is an essential reagent in transformation.

**Hypotheses:**

1. Bacteria in Luria Broth with Lux plasmid undergo lawn growth and do not demonstrate illumination

2. Bacteria in Luria Broth with Lux plasmid and antimicrobial peptides (Amp) undergo colonial growth and demonstrate illumination

3. Bacteria in Luria Broth with antimicrobial peptides (Amp) and no plasmid undergo no growth at all

**Methods**

**Preparation of competent cells**

630 μL solution of CaCl2 was introduced to the 50 μL bacterial solution using sterile pipe. After that, mixture was allowed to incubate in ice for 10 minutes. Due to this practice bacterial cells become competent; more open for up-taking foreign DNA.

**Uptake of DNA by component cells**

 Two Eppendorf tubes were labeled as C (for control plasmid) and lux (for lux plasmid) and 5 μL solution of each plasmid was placed in the prospective tubes. 70 μL competent bacterial cell solution was poured in each tube using sterile transfer pipette and kept in ice for ten minutes. Meanwhile, “no plasmid” tube was prepared using 35 μL competent cell solution only. A water bath maintained at 37° temperature was introduced with all the tubes and allowed to set for 5 minutes. 275 μL nutrient broths were added in all the three tubes and were allowed to incubate at 37° for 45 minutes.

**Identification of cells who have taken up the plasmid**

130 μL solution of each of the three tubes (control, lux and no plasmid) was poured into the three separate agar plates. A cell spreader dipped and flamed in ethanol lamp was used to spread the mixture on agar plate. Plates were covered with lids, kept for 10 minutes, inverted and incubated at 37°.

**Examining culture in the dark:**

Each tube was removed from the refrigerator. After removing lid, it was observed whether bacteria are grown in the medium or not and if they are grown, which sort of growth patterns they have. Bacterial illumination was observed keeping the plates in the dark room and transformation efficacy was calculated.

**Table 1.0**

*Predictions*

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment  | Expected result  | Bioluminescence  | Reason for expectation  |
| LBLux | Growth, lawn | No  | Amp is not present to kill *E. coli*  |
| LB/AmpLux | Growth, colonial  | Yes  | Amp will be deactivated by plasmids  |
| LB/AmpNP | No growth  | No | Amp will kill E. coli because there are no plasmids |

**Results**

Results of the above mentioned laboratory activity were as follows:

1. Bacteria underwent lawn growth in Luria Broth with Lux plasmid and did not demonstrate illumination
2. Bacteria underwent colonial growth in Luria Broth with Lux plasmid and antimicrobial peptides (Amp) and demonstrated illumination
3. Bacteria underwent no growth at all in Luria Broth with antimicrobial peptides (Amp) and no plasmid

**Table 2.0**

*Results*

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment  | Result  | Bioluminescence  | Reason  |
| LBLux | Growth, lawn | No  | Amp is not present to kill *E. coli*  |
| LB/AmpLux | Growth, colonial  | Yes  | Amp is deactivated by plasmids  |
| LB/AmpNP | No growth  | No | Amp killed E. coli because there are no plasmids |

**Discussion**

Bacteria underwent lawn growth in Luria Broth with Lux plasmid and demonstrated illumination because there were no antimicrobial peptides to kill them and Lux operon genes were incorporated due to which they were supposed to demonstrate illumination when observed in dark room but actually did not show illumination because of lawn growth (Tang et. al., 1994).

Bacteria underwent colonial growth in Luria Broth with Lux plasmid and antimicrobial peptides (Amp) and demonstrated illumination because Ampicillin resistant bacteria allowed them to deactivate antimicrobial peptides which made their survival possible. Illumination was due to the lux operon plasmid which is responsible for producing proteins that assist light reactions (Inoue, Nojima, & Okayama, 1990).

Bacteria underwent no growth at all in Luria Broth with antimicrobial peptides (Amp) and no plasmid because they were not inoculated with plasmids having ampicillin resistant genes and hence were killed by antimicrobial peptides (Amp) (Tang et. al., 1994). All the hypotheses formulated before starting experiment were verified by the results along with the predictions.

Transformation efficiency is the efficiency of cells through which extracellular DNA (through plasmid) is taken up by the host cells (bacteria) (Tang et. al., 1994). It is directly proportional to the cell competence and is calculated through dividing the number of colonies by the actual amount of DNA used. This may range from 1 ×102 cfu/μg to 1× 1011 cfu/μg in case of plasmids (Mercenier & Chassy, 1988).

**Transformation efficiency = Total no. of colonies on the LB/AmpLux plate**

 **Total amount of DNA spread on the LB/AmpLux plate**

 = 10

 130

 **Transformation efficiency = 1.3 × 103 cfu/μg**

Hence, transformation efficiency for each plate with colonial growth can be determined using above formula.

Various factors affect the transformational efficiency of bacterial cells including plasmid size, cell genotypes, forms of DNA, methods of transformation, growth of cells, and damage to DNA (Ymer, 1991; Inoue, Nojima, & Okayama, 1990; Hanahan, Jessee, & Bloom, 1991; Tang et. al., 1994; Gründemann & Schömig, 1996; Chen, 2004). Such factor must be addressed carefully while performing laboratory experiments.

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