

Gene expression analysis report examples

[Science](#), [Genetics](#)



Introduction

Gene expression is the process through which the information that has been encoded in the gene is firstly converted into messenger RNA and then finally to a protein. The study of gene expression is critical especially to the biological student and more so to one who would like to study biology related course like microbiology in future. Therefore, it is important to note that we study gene expression in order to determine how the genes and the proteins which they encode functions in the intact organism. Zhang (2006), Another reason why we study gene expression is in order to enable us decipher the functions of genes and their protein products. Gene expression also enables scientists to have a closer picture of the complex regulatory networks of genes which controls various fundamental biological processes in the body. Hussein (2007).

Various techniques have been employed by scientist in the study of gene expression. Some of the well developed and well-established and which were recently developed have used cell extracts or cultured cells in an attempt to study gene expression and function. However, some of the techniques used to determine gene expression include reporter gene, Northern blot, Western blot Fluorescent in situ hybridization, reverse transcription PCR. The higher-plex techniques include SAGE, DNA microarray, Tilling array and the RNA sequencing.

Some of the important techniques used so far include the PCR technique and the RT-PCR technique. The RT-PCR nowadays has become a standard for the detection and quantification of the RNA targets and the northern blot. All these techniques aim at determining the expression of genes. It is also

important to note that most students have not always been able to establish the difference existing between these two techniques. It is important to note that while RT-PCR is often used to qualitatively detect the gene expression through the creation of complimentary DNA transcript from RNA, PCR is used to quantitatively measure the amplification of the DNA by using the fluorescent probes. Ling and Lee (2004).

The techniques that have been applied in this experiment include reverse transcription PCR and the RNA sequencing. These are the main techniques that have been used in this study as shown in the results.

METHODS:

This part of the paper will describe some of the methods and used to determine ways through which genes express themselves. It is prudent to note that two main techniques for gene expression were used in this study. These techniques include the RT-PCR and the PCR techniques. In an attempt to study gene expression. The RNA was extracted from cell under room temperature and pressure. The following procedure was used while extracting RNA from the cells.

The first procedure done was to centrifuge the Ramos cells for five minutes then finally discard the supernatant. The next procedure done was to add 600µl of RTL lyses buffer and then pipette up and down several times to destroy the plasma membrane after which 700µl of 70% Ethanol was prepared to the tube and then added in the tube. About 700µl of the mixture is then taken to an RNeasy column and spin for 15 seconds at 10, 000 rpm. Discard the supernatant after which you add RPE buffer to the column and then spin for 15 seconds at 10, 000 rpm. Discard the flow. through. (1st

wash). Add 500µl of RPE buffer to the column and then spin for 15 seconds at 10, 000 rpm. Discard the flow through. (1st wash). The next step was to repeat step 7 by adds another 500µl of RPE buffer and centrifuge for 2 minutes at 10, 000 rpm. The next step is to Transfer all contents from the column to a clean tube and centrifuge for 1 minute at 14, 000 rpm. Place the tube to a new tube and then add 30µl of water. Spin for 1 minute at 10, 000 rpm. “ Doing this step to elute the RNA.” And then finally the last step was in Place the tube to a new tube and then adds 30µl of water. Spin for 1 minute at 10, 000 rpm. “ Doing this step to elude the RNA.” After this series of steps, the RNA was ready to be quantified using a spectrometer and its expression determined.

RT-PCR: Reverse transcriptase polymerase chain reaction (RT-PCR).

This was also another technique used in this experiment in order to determine the expression of the genes. In order to analyze how genes express themselves using this technique, the following procedure was followed.

About 0. 4 µl of 0. 1µg of isolated Ramos RNA was taken. About 0. 6µM of each of the reverse and forward primers was added to the reaction. Then 400µM of the dNTP and Add 1X RT-PCR buffer was added to the already common tube. The reaction was then completed using complete reaction mix by using ddH₂O to become to the final volume of 50µl. In order to make the results obtain here authentic and reliable, control experiments were used in order to make the screen affoedabk3a rived at For the negative control will

shall use the same reaction components except the RNA sample's volume and will complete the reaction by adding ddH₂O.

Real time PCR

This technique aims at demonstrating the rapid and quantitative nature of the PCR. Here, the author will set up a dilution series of known concentrations and then eventually perform a 10 fold dilution series using the four different DNA samples for a standard curve. Each group for this case will run a standard curve for the one of those DNAs. Secondly, the groups will use the dilution curves in order to determine the relative gene expression levels for the two unknown DNA samples. It is important to note that despite of the fact that we shall be using four different primer sets and that each group is to compare the gene expression level of a single gene between their two unknown DNA samples, the class as a whole will have to examine the expression levels of the four different genes which include EGFR, Her2/Erb2, ErbB3, ErbB3 and ErbB4.

RESULTS

The following are the results obtained from the experiment. In this part of the paper, the results shall be analyzed by drawing the melting curve, the ct value report and also determination of gene expression levels for each gene under different conditions shall be analyzed. Comparing the results for the RNA Transcription of the OD 260 and the OD 280, I realize that as the time increases, the RNA transcription for the OD260 increases more compared to the RNA expression of the OD 280.

The reverse transcriptase PCR is a technique used to determine the gene

expression in the cells of the living organism. It is prudent to note that while RT-PCR is often used to qualitatively detect the gene expression through the creation of complimentary DNA transcript from RNA, PCR is used to quantitatively measure the amplification of the DNA by using the fluorescent probes.

2. RNA gel:

M= Marker (control)A= My sample

3. RT-PCR gel:

M= Marker (control)A= My samples

4. Real Time PCR: Table of Ct values:

HER2/ErbB2

DISCUSSION

In this chapter of the paper, I shall discuss some of the differences between the PCR and the reverse transcriptase PCR. Most of the time, most student do confuse this. It is prudent to note that while RT-PCR is often used to qualitatively detect the gene expression through the creation of complimentary DNA transcript from RNA, PCR is used to quantitatively measure the amplification of the DNA by using the fluorescent probes. Other results that were observed in the RT-PCR include the changes in the expression of genes as a result of the change in temperature. Fluorescent was also observed during the experiment. Hung and Ma (2006).

It is prudent to note that the reverse transcriptase –polymerase chain has the ability to show how various genes express themselves. However, the validity of the results obtained as specific evidences of the specific gene expression solely depends on the use of the appropriate controls. For

instance, the negative control is always designed in order to demonstrate the absence of the PCR products in amplifications of the c DNA especially from the cells which do not express the gene and in reactions that do not contain the DNA template such as the water blanks. On the other hand, positive evidence should be able to provide evidence that the c DNA template is of good quality for the desired amplification, if the gene in question is expressed by the tested cells. Hussein (2007).

The q PCR nowadays represents a method of choice that is widely used by the scientist to analyze the gene expression of a moderate number of genes anywhere from a small number to thousands of samples. The end point q PCR requires that the PCR products be detected and then quantified by the gel electrophoresis after the completion of the reaction. On the other hand, it is important to note that RT-PCR remains the gold and the most important technique for studying the gene expression. Jordan (2001).

The end point of the RT-PCR does not require that its products be detected and quantified by the gel electrophoresis especially after the completion of the reaction. RT-PCR is often used to qualitatively detect the gene expression through the creation of complementary DNA transcript from RNA while q PCR is often used to quantitatively determine the expression of the genes. Patterson (2007).

In conclusion, I would like to state that caution should be taken when interpreting the negative RT-PCR results especially when the positive control amplification tests is not c DNA specific and single gene specific. Balbás and Lorence (2004).

References

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