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AMPLIFICATION OF WHOLE mRNA IN SMALL AIRWAYS EPITHELIAL CELL BY TEMPLATE SWITCING METHOD

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CHAPTER 1

1.0 INTRODUCTION

1.1 Background of Study

Small airways epithelial cells

The small airways epithelial cells are vital part of the lung that are positioned at the interface between conducting airways and alveoli (Lilly et al., 1997). In the airways, they orderly aligned forming a continuous lining as protection or barrier against exogenous agents which can be lethal to the lung that are mainly result of inhaled substance for instance particulate pollutant and also pathogens (Picot, 2005). Providing sustain conduit for air from and also to the alveoli, site where diffusion or gases exchange occur is another role plays by this cell. Generally, small airways epithelial cell is made up of cuboidal and columnar type of cell. This cell is one of the major determinant and predominant area of interest for researchers in patient having diseases which are related or closely related to lung such as bronchiolitis obliterans and chronic obstructive pulmonary disease as a comparison to normal small airways epithelium (Crystal et al., 2008).

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a technique developed by Kary Mullis (Bartlett and Stirling, 2003) that is being used for amplification of nucleic acid that involves the utilization of primer and oligonucleotides in order to amplify certain DNA or RNA fragment of interest (Pelt-Verkuil et al., 2008). PCR is comprised of three steps which are denaturation, annealing and elongation step. For the first step which is denaturation, it requires the unwinding of double stranded DNA into separate entity forming two single stranded nucleic acid by heating the DNA sample at a certain temperature and duration of time in aqueous condition. The most common temperature used is 94 °C for a period of as low as 30 seconds and up to an extent of 5 minutes. Annealing step is a process of primer binding to each single stranded DNA by hydrogen bonding and adjustment of temperature to a range between 40 °C and 65°C of the reaction mix is necessary for this reaction or process to be carried out. The adjustment of the temperature might be different in each condition that depends solely on the design of the primer. This step is also known as primer hybridisation step. The temperature is then once again being increased to about 72°C which is also an optimum temperature for polymerase-mediated DNA strand replication of heat-tolerant DNA for the last step, elongation step (Pelt-Verkuil et al., 2008). In this step, double stranded DNA or also known as amplicon will be formed as taq DNA polymerase will add the oligonucleotide in 5' to 3' direction which must be complementary to the DNA template (Chaitanya, 2013). This cycle then is being repeated by restarting from the denaturation step to keep on increasing the number of DNA of interest for few times as a typical PCR program commonly carry out around 30 to 50 of amplification cycles which is just an approximation as the exact