UNIVERSITI TEKNOLOGI MARA

OPTIMIZATION OF DNA EXTRACTION FOR OBESITY STUDY

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ABSTRACT

Quality and quantity of DNA is crucial for downstream analysis in nucleic acids research especially with raising interest in pharmacogenomics study. Commercially available DNA extraction kit would include protocols and procedure for the kit, however it could be optimized through adjustment of certain parameters to obtain better yield and greater quality of DNA. The aim of this study is to improve quality and quantity of DNA obtained. For that, adjustments were made on centrifugation speed and incubation time to determine their influence on DNA yield. Standard procedure for centrifuge speed was 14000 rpm for 1 minute was modified to 7000 rpm for 20 minutes and incubation time was increased twice the time of suggested protocol from Promega. It was observed that yield of samples processed with modified procedure was statistically significant increase from a mean of 35.22 ng/ml to 69.97ng/ml. Even with statistically significant data (p=0.49<0.05), a conclusive remarks could not be generated due small sample size (n=6). In order to validate the data, the sample size should be increased and several centrifugation speeds are chosen to further optimize DNA extraction.

CHAPTER 1

1.0 INTRODUCTION

Pharmacogenomics is growing and has gathered lots of interest in medical and health field through its high association with diseases and treatment (Kalow, 2006). It is unavoidable that the use of deoxyribonucleic acid (DNA) is crucial in genetic related studies. DNA plays an important role as it is one the main characters in genetic research. In nucleic acid study, the quality and quantity of DNA obtained from sample is vital for research as the obtained DNA should be within desired range of specification for it to be applicable in downstream or upstream application.

Isolation of DNA is necessary for genetics related analysis which will then be used for research and various purposes. As DNA is located in the nucleus of cell, the sources of sample will determine whatever stages are compulsory for extraction. This is due to the fact that presence of other compounds such as proteins, lipids, or inorganic compounds interferes with analysis method such as polymerase chain reaction (PCR). Extraction of DNA can be from any living or dead organism as long as it has genetic component.

Centrifugation speed influences sedimentation speed of materials, that substances with high molecular weight sediments faster than small one (Burgi & Hershey, 1963; Hutchinson & Krasin, 1976). Extension of centrifugation time would allow more time for DNA to sediment and contribute to increase its yield (Golder, 1953). This