Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 21, No. (4) : 2019 : 920-923

ISSN-0972-3005

IDENTIFICATION RS9642880 TAQMAN GENOTYPING USING LIGHTCYCLER 480

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(Received 6 February, 2019; accepted 3 April, 2019)

Key words: *SNP Genotyping Thermo Fisher, Light Cycler 480, rs9642880, Bladder cancer*

Abstract – We have identified the SNP Genotyping kit rs9642880 manufactured by Thermo fisher using the Roche Light Cycler480 qPCR instrument. Generally, qPCR instrument systems can only be utilized for reagent kits manufactured by the same company, but with a few modification procedures, the ThermoFisher SNP Kit can be applied for the LightCycler 480 instrument. The selection of rs9642880 is due to an increase in bladder cancer cases in Indonesia. This study successfully identified Thermo Fisher SNP kit by LightCycler480 and the average genotype found in the student respondents was heterozygous GT by 48%.

INTRODUCTION

Generally, companies that produce polymerase chain reaction (PCR) or quantitative PCR (qPCR) devices will also produce particular reagents that can only be utilized for the device. Therefore, utilization of unparticular reagents was unsuitable. However, available qPCR devices with open system tool created by some companies recently let researchers apply unparticular reagent from various companies. We reported the utilization of TaqMan SNP Genotyping reagents product of ABI-ThermoFisher ® in identifying bladder cancer using qPCR LightCycler480 instrument from Roche ®.

Bladder cancer is one of the most common malignant cancers found worldwide. In Indonesia, the disease is among the top ten malignant diseases in men with an incidence rate increase in 15% per year in the last decade (Umbas, 2008)(Umbas, Mochtar and Rahardjo, 2011)(Monoarfa and Tjandra, 2016).

Early detection in molecular biology is one of the preventive measures against the onset of cancer. Early detection at genetic level in Europe and America is a routine procedure to do, but is still rarely performed in Indonesia due to limitation of special facilities and infrastructure.

Several studies reported that mutations in a chromosome area 8q24 with SNP number rs9642880 have a susceptibility to the emergence of bladder cancer (Zhang *et al.*, 2014); (Wang *et al.*, 2018); (Cortessis *et al.*, 2010); (Lambertus *et al.*, 2015); (Freedman *et al.*, 2006). The genotype variant of GT and TT has an important role in increased risk of bladder cancer compared to GG genotype (Wang *et al.*, 2018); (Lambertus *et al.*, 2015); (Sun *et al.*, 2015). A genetic detection in medical students was conducted in this study, in order to do early prevention of cancer by way of a healthy lifestyle.

MATERIALS AND METHODS

One hundred and two blood samples were taken from medical students of UIN Jakarta. Subjects involved were previously asked for their willingness to be the subject of research by signing an informed consent. Three milliliters of EDTA blood were stored at minus twenty degrees Celsius until time to isolate the blood genome.

Genomes were isolated using Genomic DNA Mini Kit. All samples were measured for purity and concentration using Nanodrop DenNovix ® DS-11+Spectrophotometer. Two microliter diluted DNA samples (1:4) were added in eight microliters of Mix SNP Solution (containing Mix Assay (1:1), Mix buffer, and water) from TaqMan SNP Rs9642880

Thermo-fisher®. Amplification using Light Cycler 480 from Roche ®, with program steps as shown in table 1 was performed.

Detection format: dual color hydrolysis/UPL Probe and Analysis by Endpoint Genotyping.

RESULTS AND DISCUSSION

Genomic isolation of one hundred and two respondents from medical students were carried out, which consist of thirty-six males and sixty-six females. A qualitative analysis using gel-agarose electrophoresis technique was performed in order to determine the success of genomic isolation while spectrophotometer technique was applied to measure the concentration and purity of each genome. The mean purity and concentration of the genome DNA is 1.85 (A 260/A 280) and 133.7 ng/uL respectively.

The genotype results of SNP rs9642880 screening for Hydrolysis Probe Endpoint Genotyping technique from TaqMan SNP Genotyping kit using LightCycler 480 machine from Roche were 35.3% GG, 48% GT and 16.7% TT. There was no significant difference between genotypes and gender (Table 2). The percentage of T and G alleles on all respondents was 41:59, and there was no significant difference between alleles and gender (Table 3).

In this study, Rs9642880 screening was performed utilizing TaqMan SNP Genotyping Kit manufactured by ThermoFisher Company. Light Cycler 480 produced by Roche Company was successfully applied for read and analyzed the result. Generally, TaqMan kits are only used with Applied Biosystems (ABI) 7300 PCR System Machine. We have carried out a preliminary test using ABI 7300 in another laboratory (SEAMEO Lab, Faculty of Medicine University of Indonesia) and did not give different result using Light Cycler 480.

A single nucleotide polymorphism or SNP is the most common form of genetic variation in species including humans. These differences between individual bases of DNA often do not directly affect gene expression, but in many cases, it can still be useful for searching and diagnosing patients with diseases related gene (BCAN, 2012)(Twyman, 2009). Studies from the Genome-Wide Association (GWA) have reported a correlation of *cMYC* gene with bladder cancer. cMYC is a multifaceted protein that regulates cell proliferation, differentiation and apoptosis. SNP Rs9642880 is a single polymorphism found in the cMYC gene on chromosome 8, encoded 8q24, and is assured to regulate fifteen percent of all genes through the bonding of E-Boxes and HATs. The change of nucleotide G to T (G>T) in Rs9642880 causes an individual's susceptibility to bladder cancer and is also associated with the *CASC11* gene that has a link in the process of cancer occurrence (Lambertus *et al.*, 2015)(Freedman *et al.*, 2006) (Nilsson and Cleveland, 2003)

Bladder cancer is one of the most common

Table 1. Light Cycler ® Software release 1.5.1.62SP1

			Program			
Name	Cycles	Analysis Mode	Target ^{0}C	Acquisition Mode	Hold Hh:mm:ss	Ramp rate
UNG	1	None	50	None	00:02:00	4.4
Poly Act	1	None	95	None	00:00:20	4.4
PCR	50	Quantification	95	None	00:00:03	4.4
			60	Single	00:00:20	2.2
Coll	1	None	40	None	00:00:30	2.2

Table 2. Correlation between genotype and genders

Gender's	Genotype						Total		*P* value
	GG		GT		TT				
	N	%	N	%	N	%	N	%	0.720
Male	11	30.6	18	50.0	7	19.4	36	100	
Female	25	37.9	31	47.0	10	15.2	66	100	
Total	36	35.3	49	48.0	17	16.7	102	100	

No Significant between genotype and genders

malignant neoplasms. In 2012, mortality rates due to bladder cancer increased in China. In the United Kingdom, 1:3 mortality rates from bladder cancer. Smoking is the most common risk factor found in patients, in addition to ethnic and age factors (Sherman, 2013)(Globocan, 2012).

Bladder cancer cases data onto Indonesia is still not well recorded, meanwhile the number of active smokers in Indonesia quite a lot, especially in men. Seeing the number of active smoker, and not well-recorded case, it is necessary to prevent the occurrence of bladder cancer, one of them by listing the SNP on the group of productive individuals in Indonesia. By knowing the SNP on the individual, it will be easier to provide information in terms of prevention. In this study, we found that nearly 50% of respondents had heterozygous genotypes for Rs9642880 (Figure 1) (Table 2), where GT genotypes were 1.2 times the risk of genotype GG for bladder cancer (Sun *et al.*, 2015; Jemal Bray and Ferlay, 2011) The frequencies of the risk allele (T) were not significantly different between male and female implying that all genders have the same risk probability to have bladder cancer genetically (Figure 2) (Table 3). However, bladder cancer is more common among men than women (Davis-dao *et al.*, 2012). Non-genetic factors might have a role in increasing the risk of bladder cancer in men.

The conclusion of our research is the identification of single mutation with SNP Genotyping Kit from Thermofisher can be performed utilizing the Light Cycler 480 instrument with little modification on the DNA fragment amplification program and detection of mutation area.

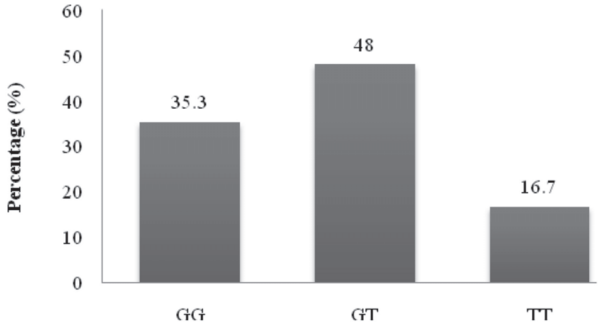

Fig. 1. Percentage distribution of genotyping rs942880 medical students 2012

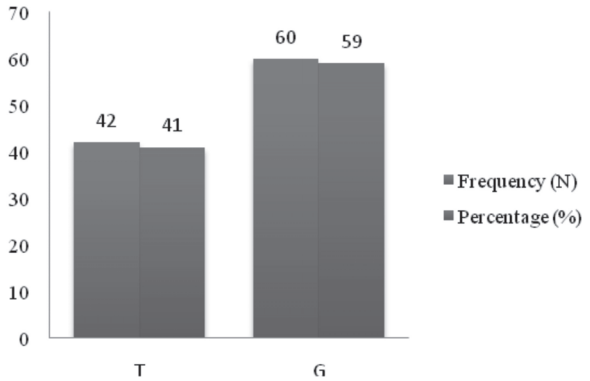

Fig. 2. Frequency and percentage of allele T and G on all respondents

ACKNOWLEDGMENT

We would like to thank all the respondents that have been willing to participate in this research. We also thank the Faculty of Medicine and State Islamic University (UIN) Syarif Hidayatullah Jakarta who have provided assistance in funding.

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Table 3. Association between allele and genders

Allele	Gender's				Total		*P* value
	Male		Female				
	N	%	N	%	N	%	
T (risk allele)	16	44.4	26	39.4	42	41.2	0.652
G	20	55.6	40	60.6	60	58.8	
Total	36	100	66	100	102	100	

Not significant between allele and genders

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