SUBCLONING OF CYTOCHROME P450 2C19 (CYP2C19) IN *ESCHERICHIA COLI* STRAIN BL21

by

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LIST OF ABBREVIATIONS

$CaCl_2$	calcium chloride
cDNA	complementary DNA
DNA	deoxyribonucleic acid
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
dH_2O	distilled water
dNTP	deoxynucleoside triphosphate
dTTP	deoxythymidine triphosphate
EDTA	ethylenediaminetetraacetic acid
EtBr	ethidium bromide
EtOH	ethanol
E. coli	*Escherichia coli*
gDNA	genomic deoxyribonucleic acid
HCl	hydrochloric acid
H_2O	water
KCl	potassium chloride
LB	Luria-Bertani
$MgCl_2$	magnesium chloride
$NaCl_2$	sodium chloride
NaOAc	sodium acetate
$(NH_4)_2SO_4$	ammonium sulfate
NCBI	National Centre for Biotechnology Information
OD	optical density
ORF	open reading frame
PCR	polymerase chain reaction
RE	restriction enzyme
RNA	ribonucleic acid
sec	seconds
Ta	annealing temperature
TAE	tris-acetate EDTA

TBE	tris-borate EDTA
Tm	melting temperature
UK	United Kingdom
USA	United States of America
UV	ultraviolet
v/v	volume to volume
WHO	World Health Organization

LIST OF SYMBOLS

~	approximately
%	percent
'	prime
Ω	Ohm
°C	degree celcius
bp	base pairs
g	gram
k	kilo
kb	kilo base
µl	microlitre
U	unit
mg/ml	miligram per mililitre
ml	mililitre
mM	milimolar
ng/ µl	nanogram per microlitre
nm	nanometer
pmol/ µl	picomole per microlitre
rpm	revolution per minute
X	times
x g	times gravity

ABSTRAK

Metabolisme atau biotransformasi ubat mengalami perubahan molekul dimana, menukarkan molekul yang tidak polar, molekul ubat lipofilik yang aktif secara farmakologi kepada metabolit polar, tidak aktif, atau tidak beracun dalam tubuh sebelum dikumuhkan. Dalam manusia, cytochrome P450 (CYP) enzim adalah pemangkin utama yang terlibat dalam metabolisme ubat-ubatan dan xenobiotik lain yang memasuki tubuh. Enzim CYP2C19 bertanggungjawab untuk metabolisme ubat seperti antidepresan. Penelitian ini dilakukan untuk menghasilkan CYP2C19 enzim rekombinan untuk kajian pharmakogenomik. cDNA dihasilkan dari CYP2C19 dan diperolehi daripada Origene, USA. Proses pengklonan dilakukan dengan menggunakan RapidShuttling ™ Kit (Origene, USA). CYP2C19 dari TrueORF Entry Vector dipindahkan ke vector ekspresi, pEX-C-His. Plasmid yang di ingini diekstrak dengan menggunakan komersial QIAprep Spin Miniprep Kit dan semakan jujukan DNA CYP2C19 disahkan dengan penjujukan DNA menggunakan ABI PRISMA™ 3130xl Genetyx Analyzer. Saiz produk Tindakbalas Polimerase Berantai (TPB) pada gel agarosa adalah pada ~1.5kb. Jujukan DNA enzim CYP2C19 yang di ingini adalah sama seperti di Gene bank. Sebagai kesimpulan, kajian menunjukkan gene *CYP2C19* bejaya diklonkan. Hasil gabungan biologi molekul dan pendedahan relative kepada substrat CYP2C19 dan ujian genetik dapat memberikan kesan positif untuk mengelakkan hasil tindak balas toksikologi yang dihasilkan oleh enzim dan variasi yang ditunjukkan.

ABSTRACT

Drug metabolism or biotransformation of drug undergo molecular alteration in which, it converts nonpolar, lipohilic pharmacologically active drug molecules into polar, inactive, or nontoxic metabolites in the body before excretion. In human, the cytochrome P450 (CYP) enzymes are the major catalysts involved in the metabolism of drugs and other xenobiotics that enter the body. The CYP2C19 enzyme is responsible for the metabolism of several important drugs such as antidepressants. This study was conducted to generate CYP2C19 enzyme recombinant for pharmocogenomics study. Constructed cDNA of CYP2C19 was purchased from Origene, USA. The cloning process was performed by using RapidShuttling™ Kit (Origene, USA). The *CYP2C19* gene from TrueORF Entry Vector was transferred into an expression vector, pEX-C-His. The desire plasmid was extracted using commercial QIAprep Spin Miniprep Kit (Qiagen, Germany) and DNA sequence of CYP2C19 was confirmed by DNA sequencing using ABI PRISMA™ 3130*xl* Genetyx Analyzer. The size of PCR product was shown at ~1.5kb on agarose gel. Desire DNA sequence of CYP2C19 enzyme shown same sequence as in Gene bank. As a conclusion, *CYP2C19* gene was successfully cloned. The combination of molecular biology and relative exposure to CYP2C19 substrates and genetic testing eventually lead to a better appreciation to avoid toxicological outcomes that may currently result from this enzyme and variations in its expression.