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Optimization of PCR Conditions for Amplification of GC-Rich *EGFR* Promoter Sequence

Jasmina Obradovic,[1,2] Vladimir Jurisic,[1] Natasa Tosic,[3] Jasminka Mrdjanovic,[4] Branislav Perin,[5] Sonja Pavlovic,[3] and Natasa Djordjevic[1]*

[1]*Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia*
[2]*Faculty of Science, Institute of Biology and Ecology, University of Kragujevac, Kragujevac, Serbia*
[3]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia*
[4]*Oncology Institute of Vojvodina, Faculty of Medicine, University of Novi Sad, Sremska Kamenica, Serbia*
[5]*Institute for Pulmonary Diseases of Vojvodina, Clinic for Pulmonary Oncology, Faculty of Medicine, University of Novi Sad, Sremska Kamenica, Serbia*

Background: Polymerase chain reaction (PCR) is an extremely sensitive method that often demands optimization, especially when difficult templates need to be amplified. The aim of the present study was to optimize the PCR conditions for amplification of the epidermal growth factor receptor (*EGFR*) promoter sequence featuring an extremely high guanine-cytosine (GC) content in order to detect single nucleotide polymorphisms -216G>T and -191C>A. *Methods:* Genomic DNA used for amplification was extracted from formalin-fixed paraffin-embedded lung tumor tissue and PCR products were detected by agarose gel electrophoresis. *Results:* Results showed that addition of 5% dimethyl sulfoxide (DMSO), as well as DNA concentration in PCR reaction of at least 2 μg/ml, were necessary for successful amplification. Due to high GC content, optimal annealing temperature was 7°C higher than calculated, while adequate $MgCl_2$ concentration ranged from 1.5 to 2.0 mM. *Conclusion:* In conclusion, *EGFR* promoter region is a difficult PCR target, but it could be amplified after optimization of $MgCl_2$ concentration and annealing temperature in the presence of DMSO and the DNA template of acceptable concentration. J. Clin. Lab. Anal. 27:487–493, 2013.

Key words: EGFR; GC-rich; genotyping; optimization; PCR

INTRODUCTION

Polymerase chain reaction (PCR) is an enzymatic in vitro method for exponential amplification of specific DNA target sequence, affordable and suitable for both basic research and various clinical applications (1). However, the method is extremely sensitive, thus, it could be a considerable challenge to optimize the conditions of the reaction in order to obtain the desired results, especially when difficult templates, such as GC-rich regions, need to be amplified. Namely, GC-rich regions, due to formation of stable and complex secondary structures within a DNA template, could block DNA polymerase during PCR reaction and lead to an ineffective amplification (2–6). PCR technique parameters that could affect its accuracy and efficacy are numerous, including concentration of DNA template, concentration of magnesium ions, PCR thermal cycling conditions, as well as addition and concentration of PCR additives (7, 8). If there is a scientific or clinical need for specific and efficient amplification of GC-rich DNA template, tuning the PCR reaction could be highly demanding, yet, critically important.

Epidermal growth factor receptor (EGFR) expressed in several epithelial cancers, including lung, breast,

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*Correspondence to: Natasa Djordjevic, Department of Pharmacology and Toxicology, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovica 69,34000 Kragujevac, Serbia. E-mail: natashadj2002@yahoo.com

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