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TÜBİTAK UME

Effects of Different High Resolution Melting Dyes on DNA Methylation Measurements

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INTRODUCTION

Gene Methylation is a reversible, epigenetic mechanism that may change the activity of a DNA segment without changing the DNA sequence. Beside the normal biological process, it has also roles in the formation of diseases.

Normal Biological Process

- Regulation and control of gene expression
- Embryonic development
- Chromatin structure
- Inactivation of X chromosome
- Transcription
- Aging

Disease formation

- Cancer
- Diabetes
- Cardiovascular diseases
- Autoimmune diseases
- Neurological disorders

This epigenetic mechanism can be used in early diagnosis and treatment of diseases, because, it is possible to detect the differentially methylated DNA fragments in liquid biopsy samples even in early stages of the diseases.

There is an urgent need for internationally accepted gene methylation measurement system. For this purpose, Korea Research Institute of Standards and Science (KRISS) and TUBITAK National Metrology Institute of Turkey (UME) has been working on this subject with an aim of developing an accurate, reproducible and SI traceable reference measurement system.

TUBITAK UME investigated several parameters effecting gene methylation analysis using the plasmid controls designed by KRISS and developed a measurement method which includes qPCR and High Resolution Melting Curve Analysis (HRM).

In this study, we aimed to investigate different commercial HRM masters affect gene methylation measurements.

* This study is a part of KRISS and TUBITAK UME collaboration project.

RESULTS

It was observed that with HRM masters mixes other than LC480 HRM master, it was not possible to obtain same amount of methylated and unmethylated amplicons. Same amount of amplicon was only obtained using LC480 HRM master which utilize $MgCl_2$ optimisation.

In the figures 1, 2 ,3 and 4, melting peaks are given which were obtained using different HRM dyes.

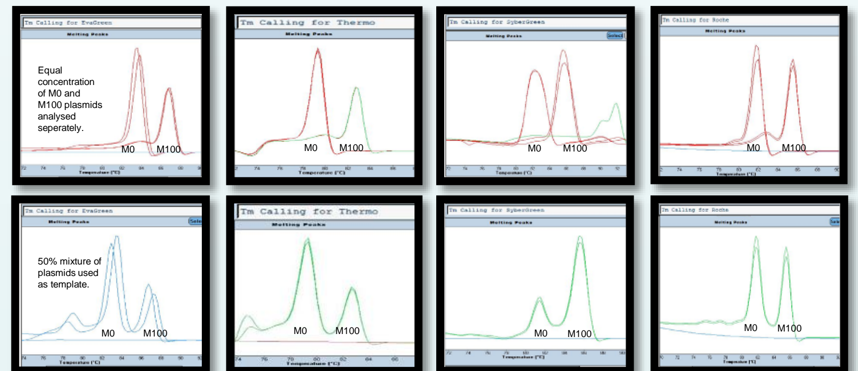

Figure 1: HOT FIREPol EvaGreen qPCR Mix Plus | Figure 2: MeltDoctor HRM Master Mix | Figure 3: SensiFAST SYBR Green | Figure 4: LC480 HRM Master

In the figures 5, 6, 7 and 8, melting peaks are given which were obtained under different $MgCl_2$ concentration of LC480 HRM Master.

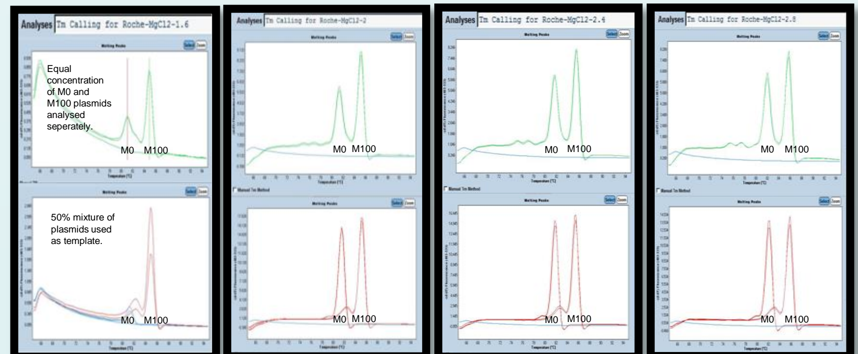

Figure 5: 2 mM $MgCl_2$ | Figure 6: 2,5 mM $MgCl_2$ | Figure 7: 3 mM $MgCl_2$ | Figure 8: 3,5 mM $MgCl_2$

MATERIAL & METHOD

Template DNA: Plasmid controls (containing bisulfite converted methylated (M100) and unmethylated (M0) sequences) were designed for RARBeta promotor region. The concentration of the plasmids were equilised using assays targeting plasmid backbone. 10^6 copy/μL M0, M100 and 50% mixture of this plasmids were used as a template.

After qPCR and HRM analysis, it was aimed to have same amount of methylated and unmethylated amplicons from the samples which has same concentration.

Commercial kits:

- LightCycler 480 High Resolution Melting (LC480 HRM) Master
- MeltDoctor HRM Master Mix
- ZymoTaq qPCR Premix
- HOT FIREPol EvaGreen qPCR Mix Plus
- SensiFAST SYBR

CONCLUSION

This study showed that;

- all the results obtained using HRM masters other than SyberGreen has an affinity to amplify unmethylated gene fragments when used 50% mixed samples
- using correct HRM dyes helps to eliminate one of the important PCR biases, that can effect the gene methylation measurement results
- $MgCl_2$ concentration has an important effect to avoide/minimize PCR bias caused by HRM master
- using controls designed specifically to a gene helps to minimize PCR bias and to optimise measurement method for gene methylation.

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