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QUANTIFICATION OF DNA METHYLATION FOR COMT, RELN AND HTR2C IN SCHIZOPHRENIA USING METHYLIGHT ASSAY

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Epigenetic is an interaction between the environment and gene and has been attributed with pathogenesis of many complex diseases such as Schizophrenia (Sz) and also has been debated for future pharmacotherapeutic approach. The purpose of this study is to optimize the MethyLight assay method for quantification of DNA methylation for Catechol-O-methyltransferase COMT, Reelin RELN and 5-hydroxytryptamine (serotonin) 2C receptor HTR2C genes and assessing 2 reference genes, (β-actin) ACTB and ALU for its use. To assess the efficiency of MethyLight assay at different percentage of DNA methylation, DNA of universal human methylated and unmethylated samples were made into serial methylation percentage and subjected to bisulfite treatment. The primers and probes of the genes were designed to cover CpG rich sites while reference genes based on suggested literature. To assess the sensitivity of the assay at different DNA concentration, the universal human methylated DNA was diluted into 1:3 serial dilutions. Both assessments were subjected to real-time PCR assay. Amplification curves for target and reference genes were plotted at acceptable Cq values. However, the assay for HTR2C, ALU and ACTB were unable to differentiate the Cq values based on the percentage of DNA methylation, whilst the amplification assay and Cq values of RELN and COMT were proportionate and able to differentiate the percentage of DNA methylation. Serial dilution of the samples showed an acceptable standard curves with R^2 range from 0.80 to 1.00. In conclusion, ALU and ACTB can be used for reference genes as both assays showed no differences of Cq values against various percentage of DNA methylation. However ALU was more preferable as the dilution series showed a better assay efficiency especially at lower concentration of DNA. The assay for COMT and RELN were sensitive and specific for detection and quantification of DNA methylation.

QUANTIFICATION OF DNA METHYLATION FOR HTR2C, RELN AND COMT IN SCHIZOPHRENIA USING METHYLIGHT® ASSAY

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Introduction

Epigenetic is implicated in the pathogenesis of many complex diseases including Schizophrenia (Sz), and could be the basis for future pharmacotherapeutic approach [1]. There are a number of evidences suggesting Catechol-o-methyltransferase (COMT) and Reelin (RELN) genes DNA methylation in the aetiology of Sz [2, 3]. However, the evidences correlating Sz with DNA methylation are mainly qualitative and involve a small number of subjects. The use of quantitative method for the assessment of DNA methylation using real-time PCR is recommended as this method is sensitive and easily modifiable for a high-throughput assay [4].

Objectives

1. To optimize the MethyLight® assay method for quantification of DNA methylation for COMT, RELN and 5-hydroxytryptamine 2C receptor (HTR2C) target genes .
2. To assess two reference genes proposed in two separate studies, ACTB [5] and ALU [6] for their use.

Methodology

The primers and probes for target genes were designed to cover CpG rich sites, while the reference genes were based on *Eads, et al., 2000* and *Olkhov-Mitsel et al., 2014* [5, 6]. To assess the efficiency of MethyLight® assay at different percentages of DNA methylation, DNA of universal methylated and unmethylated human samples *(ZYMO research, USA)* were mixed and made into serial methylation percentages of 0%, 1%, 5%, 10 %, 25%, 50%, 75% and 100%. These mixtures were then subjected to bisulfite treatment and run on the CFX96 Real-Time PCR Detection System *(Bio-Rad, USA).* The PCR reactions contained 5 µl of Sensifast Master Mix *(Bioline, UK),* 0.5 µl of probe and primer, and 2 µl of 5 ng/µl of template DNA. The PCR protocol included initial denaturation at 95°C for 5 minutes followed by 60 cycles of 95°C for 10 seconds and 60°C for 30 seconds, respectively. To assess the sensitivity of the assay at different DNA concentrations, the 100% universal methylated DNA were diluted into 1:3 serial dilution and run with the similar PCR protocol as above.

Results

Successful amplification curves were detected for target and reference genes (Figure 1). The amplification plot and Cq values of RELN and COMT were proportionate and able to differentiate with the percentage of DNA methylation. However, the assays for HTR2C, ALU and ACTB were unable to differentiate the Cq values based on the percentage of DNA methylation. Successful amplification curve was still recorded for ALU as the lowest concentration of DNA serial dilution. Serial dilution of the samples showed a linear standard curves with an R^2 range from 0.83 to 0.99 (Figure 2).

Conclusion

ALU and ACTB can be used for reference genes as both assays showed no differences of Cq values against various percentage of DNA methylation. However, ALU was preferable as the dilution series showed a better assay efficiency especially at lower concentrations of DNA. The assays for COMT and RELN were sensitive and specific for the detection and quantification of DNA methylation.

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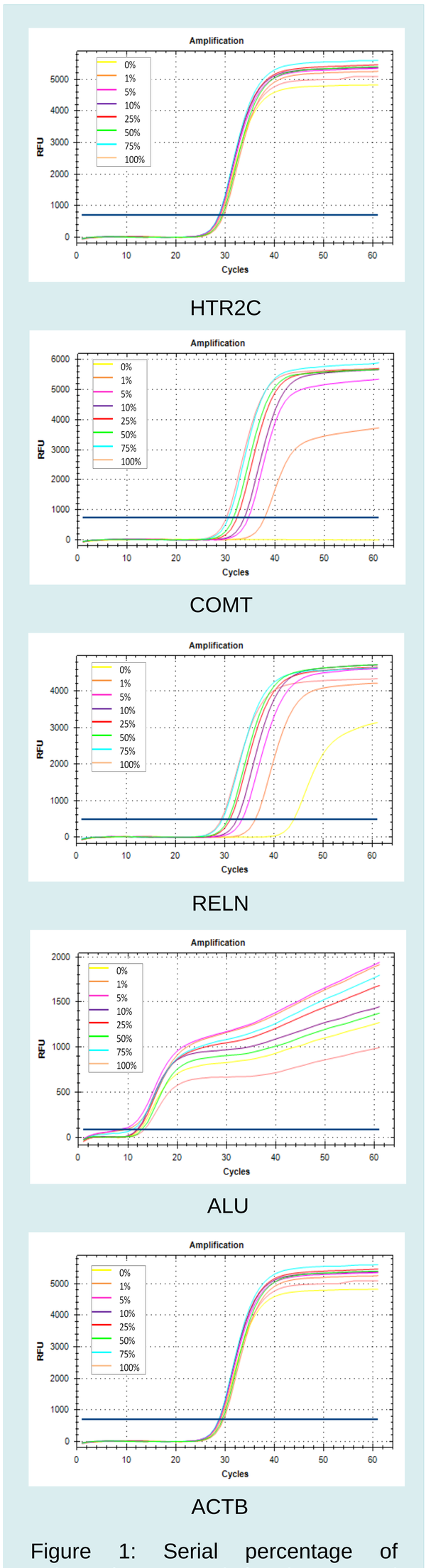

Figure 1: Serial percentage of universal methylated and unmethylated DNA of target genes (HTR2C, COMT and RELN) and reference genes (ALU and ACTB)

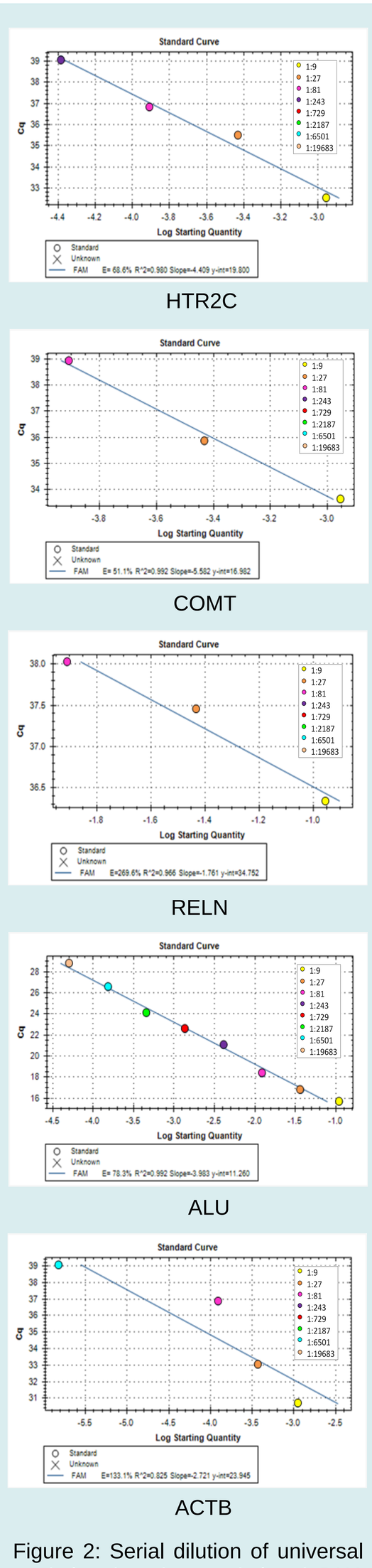

Figure 2: Serial dilution of universal methylated and unmethylated DNA of target genes (HTR2C, COMT and RELN) and reference genes (ALU and ACTB)

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