114/A

PCR amplification of the GC rich region of the FMR1 gene

C H W M R B Chandrasekera[1*], W S S Wijesundera[1] and H N Perera[2]
[1]*Department of Biochemistry & Molecular Biology, Faculty of Medicine, University of Colombo, Colombo 03*
[2]*Department of Psychological Medicine, Faculty of Medicine, University of Colombo, Colombo 03*

Amplification of the GC-rich region of the FMR 1 gene is important in screening and diagnosis of Fragile X Syndrome (FXS). FXS is due to the expansion of CGG repeats present in the 5' untranslated region of the Fragile X Mental Retardation gene1 (FMR1). Conventional PCR fails to amplify due to the high GC content. Use of expensive thermo-stable polymerases, commercial PCR enhancer solutions and DNA extraction kits have been reported in literature to improve the amplification of GC rich regions in the FMR1 gene. However, these improved procedures are too costly to be employed in laboratories of low income countries. Therefore, the main objective of this study was to develop a low cost PCR assay for amplification of the GC rich region of the FMR1 gene.

PCR optimization was carried out on DNA extracted from blood using primers designed to amplify the GC rich 5' untranslated region of the FMR1 gene. Three methods were employed in optimization; substituting a substrate analogue (7-deaza –dGTP) with varying concentrations of PCR reagents and annealing temperature, use of a single PCR additive under different concentrations and the use of a combination of PCR additives. The use of 7-deaza dGTP with varying reaction conditions and annealing temperature and the addition of 1, 2 propane diol or ethylene glycol did not promote amplification at any given concentration. The GC rich region of the FMR1 gene was optimally amplified with reproducibility for blood DNA as well as buccal cell DNA for representative samples in the presence of betaine and DMSO as additives. The PCR amplification cost was low as commercially available kits for DNA extraction or expensive thermostable polymerases were not used in the present method. Furthermore, routine screening of FXS will be facilitated by this optimized PCR conditions in laboratories with low resource settings.

Keywords: polymerase chain reaction, GC-rich sequences, fragile X syndrome, PCR additive and enhancer

chmbhagya@yahoo.com