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Usefulness of multiplex ligation-dependent probe amplification in diagnosis of Duchene Muscular Dystrophy gene mutation analysis

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The largest known gene present in humans is located on the short arm of the X chromosome (at Xp21.2), and is called the Duchenne Muscular Dystrophy (DMD) gene. DMD gene has Open reading frame ~11.055 kb and it contains 79 exons. MLPA (Multiplex Ligation-Dependent Probe Amplification) was developed as a general method to establish copy number of up to 45 nucleic acid sequences in one single reaction. This method has proved a reliable tool for the diagnosis of genetic diseases characterized by large gene deletions and duplications. This technique has made it possible to detect the entire dystrophin gene containing 79 exons in order to study the deletions and duplications in a fast and reliable manner. MLPA analysis was carried out using P034 and P035 probes purchased commercially from MRC, Holland (Amsterdam, Netherlands). The procedures were carried out according to the manufacturer's recommendations. Briefly, 100 ng DNA was denatured and hybridized overnight at 60 °C with the SALSA probe mix 034 (DMD exons 1-10, 21-30, 41-50 and 61-70) and 035 (DMD exons 11-20, 31-40, 51-60 and 71-79). Samples were then treated with Ligase 65 for 15 min at 54 °C. The reactions were stopped by incubation at 98 °C for 5 minutes. IN THE LAST Step PCR amplification was done using SALSA FAM PCR primers, and after amplification amplified products are run on Genetic analyzer ABI Prism 3100 (Applied Bio systems, USA) with the following modules: Capillaries 36 cm, Polymer POP-4, run temperature 60 °C, capillary fill volume 184 steps, pre-run voltage 15 kV, pre-run time 180 seconds, injection voltage 3.0 kV, injection time 10-30 seconds, run voltage 15 kV, data delay time 1 second and run time 1500 seconds.

Biography

Sheeba Parveen is a registered health care professional with the regulatory DHCC, Dubai. She holds a Master's degree in Molecular Microbiology from University of Karachi. She has pursued one year course on Medical Laboratory Training program exclusively in Molecular Pathology Department of Clinical Laboratory from well Known JCI, ISO and CAP accredited hospital in Pakistan. She has worked more than 11 years in the JCI, ISO and CAP accredited hospital as Molecular Specialist. She optimizes many assays including DNA sequencing, MLPA analysis for diagnosis of genetic disorders, methylation specific PCR for Prader-Willi/Angelman syndrome testing. She is also CAP Task Force Coordinator for the Molecular Pathology section of Aga Khan University Hospital, Karachi, Pakistan. She also had been involved in writing research papers and holding more than 9 local and international publications. Later, she joined Dow Diagnostic DHCC as a Molecular Specialist and served for almost 5 months and joined Pure Health as Molecular Biologist at Al Qassimi Hospital (MOH) in January 2018.

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