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Electrochemical APEX for Genotyping MYH7 Gene: A Low Cost Strategy for Minisequencing of Disease Causing Mutations

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Abstract : The completion of the human genome Project (HGP) has paved the way for mapping the diversity in the overall genome sequence which helps to understand the genetic causes of inherited diseases and susceptibility to drugs or environmental toxins. Arrayed primer extension (APEX) is a microarray based minisequencing strategy for screening disease causing mutations. It is derived from Sanger DNA sequencing and uses fluorescently dideoxynucleotides (ddNTPs) for termination of a growing DNA strand from a primer with its 3´- end designed immediately upstream of a site where single nucleotide polymorphism (SNP) occurs. The use of DNA polymerase offers a very high accuracy and specificity to APEX which in turn happens to be a method of choice for multiplex SNP detection. Coupling the high specificity of this method with the high sensitivity, low cost and compatibility for miniaturization of electrochemical techniques would offer an excellent platform for detection of mutation as well as sequencing of DNA templates. We are developing an electrochemical APEX for the analysis of SNPs found in the MYH7 gene for group of cardiomyopathy patients. ddNTPs were labeled with four different redox active compounds with four distinct potentials. Thiolated oligonucleotide probes were immobilised on gold and glassy carbon substrates which are followed by hybridisation with complementary target DNA just adjacent to the base to be extended by polymerase. Electrochemical interrogation was performed after the incorporation of the redox labelled dedioxynucleotide. The work involved the synthesis and characterisation of the redox labelled ddNTPs, optimisation and characterisation of surface functionalisation strategies and the nucleotide incorporation assays.

Keywords : array based primer extension, labelled ddNTPs, electrochemical, mutations
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