Multiplexed detection of single nucleotide polymorphisms via solid-phase primer elongation with ferrocene labelled nucleotides

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An approach for the multiplexed detection and identification of single nucleotide polymorphisms exploiting electrode arrays, primer elongation and ferrocene labelled nucleotides is presented. Solid-phase isothermal primer elongation reaction using ferrocene-labelled 2'-deoxyribonucleoside triphosphates (Fc-dNTPs) was exploited for the electrochemical detection of a single nucleotide polymorphism (SNP). Four 5'-thiolated primers, designed to be complementary with the same fragment of the target sequence and differing only in the last base at the 3-OH' end, were self-assembled with 6-mercaptohexanol on individual gold electrodes of an array. Solid phase isothermal primer elongation using Klenow (exo-) polymerase (single stranded DNA targets) for 5 minutes or isothermal recombinase polymerase amplification (double stranded DNA target) for 15 minutes at 37ºC and using an optimised ratio of Fc-dNTPs and natural dNTPs. Square wave voltammetry was used to measure the ferrocene present in the elongated primers. Elongation only occurred with the primer containing the base complementary to the single nucleotide polymorphisms present, with an unequivocal electrochemical signal observed. The platform was applied to the multiplexed detection of SNPs associated with osteoporosis using genomic DNA from a fingerprick blood sample, as well as to the detection of SNPs linked with resistance to the antibiotic rifampicin in *Mycobacterium tuberculosis* again using genomic DNA and results were validated using next generation sequencing. This generic platform has a plethora of potential applications in clinical diagnostics, detection of antibiotic resistance and forensics.

Figures

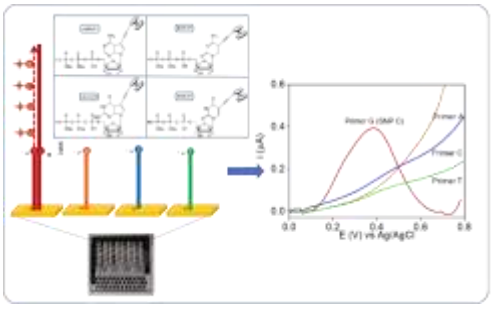

Figure 1: Schematic overview of electrochemical detection and identification of single nucleotide polymorphisms