Solid-phase isothermal primer elongation using ferrocene-labelled dNTPs for the electrochemical detection of single nucleotide polymorphisms

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The aim of this work was to develop a generic platform for the electrochemical detection of single nucleotide polymorphisms (SNPs) via isothermal solid-phase primer elongation and exploiting ferrocene-labelled 2'-deoxyribonucleoside triphosphates (dNFcTPs). Two examples of the application of this platform will be described, the first using Klenow (exo-) DNA polymerase for the detection of a single SNP linked to resistance to the antibiotic rifampicin in Mycobacterium tuberculosis. In this first example, DNA is extracted from sputum samples and amplified using asymmetric PCR to produce single stranded DNA (ssDNA). Four 5'-thiolated primers, designed to be complementary with the same fragment of the target sequence and differing only in the last base, addressing the polymorphic site, were self-assembled via chemisorption on individual gold electrodes of an array. Following hybridisation with the ssDNA, primer elongation with the dNFcTPs was only observed to proceed at the electrode where there was full complementarity between the surface-tethered probe and the target DNA being interrogated. We tested all four dNFcTPs and optimized the ratio of labelled/natural nucleotides to achieve maximum sensitivity. Following a 20 min hybridisation step, Klenow (exo-) DNA polymerase mediated primer elongation at 37ºC for 5 minutes was optimal for the enzymatic incorporation of ferrocene-labelled nucleotide, achieving an unequivocal electrochemical detection of a single-point mutation in 14 samples of genomic DNA extracted from Mycobacterium tuberculosis strains. In the second example, the use of isothermal recombinase polymerase amplification as an alternative to Klenow (exo-) DNA polymerase was pursued, with this approach avoiding the need for previous amplification and generation of single stranded DNA. Here, a SNP associated with hypertrophic cardiomyopathies (HCM), in the Myosin Heavy Chain 7 (MYH7) gene, was detected directly in fingerprick blood samples. HCM is the principal cause of sudden cardiac death in young athletes and it is estimated that 1 in 500 people have HCM, and a cost-effective tool for the screening of young athletes would assist in an early diagnosis and subsequent medical intervention. Again, four thiolated reverse primers containing a variable base at their 3' end were immobilised on individual gold electrodes of an array. Following hybridisation with target DNA, solid phase recombinase polymerase amplification was carried out and primer elongation incorporating the dNFcTPs was only detected at one of the electrodes, thus facilitating identification of the SNP under interrogation. The assay was applied to the direct detection of the SNP in fingerprick blood samples from eight different individuals, with the results obtained corroborating with next generation sequencing. The ability to be able to robustly identify the SNP using a 10 µL fingerprick sample, demonstrates that SNP discrimination is achieved using low femtomolar (ca. 8 x 105 copies DNA) levels of DNA. Finally, this second approach was applied to the simultaneous, multiplexed detection of SNPs linked with rifampicin and isoniazid resistance and the results validated with real samples genotyped using next generation sequencing.

Acknowledgements This project has received partial funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 767325 and by Czech Science Foundation (20-00885X to M. H.).