Characterization of genomic SNP via colorimetric detection using a single gold nanoprobe

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Abstract

Identification of specific nucleic acid sequences mediated by gold nanoparticles derivatized thiol-modified oligonucleotides (Au-nanoprobes) has been proven a useful tool in molecular diagnostics [1,3-5]. Here, we demonstrate that, upon molecular optimization, detection may be simplified and results attained using a single Au-nanoprobe to detect SNP in homo- or heterozygous condition (Figure 1). Au-nanoprobes are becoming extremely useful tools for routine molecular diagnostics involving single-base mismatch detection that can be a fast, cheap and reliable alternative to standard techniques [4].

We demonstrate the robustness of this approach through validation using clinical samples and screening for the SNP rs9939609 in the FTO gene locus [2]. For the first time, we demonstrate that, upon comprehensive optimization, a single Au-nanoprobe may be used alone to detect SNP by presenting distinct threshold for each genetic status (wild type, heterozygous and mutant) with high degree of sensitivity (87.50%) specificity (91.67%) [5].

Results were validated using Sanger sequencing as gold standard. Sensitivity, specificity and limit of detection (LOD) were determined and statistical differences calculated by one-way analysis of variance (ANOVA) and a post hoc Tukey's test to ascertain whether there were any differences between Au-nanoprobe genotyped groups. From the 20 samples genotyped via Sanger sequencing, 8 samples were wild type (T/T), 7 samples heterozygous (T/A) and 5 samples mutated (A/A) [4]. Genotyping using the Au-nanoprobe determined 9 wild type (T/T) samples, 7 heterozygous (T/A) and 4 mutated (A/A). The LOD for Au-nanoprobe FTOwt20 was set at 20 µg ml^{-1}.This simple and fast approach requires low complexity apparatus (UV/Vis spectroscopy) but may also been evaluated by the naked eye.

References

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Figures

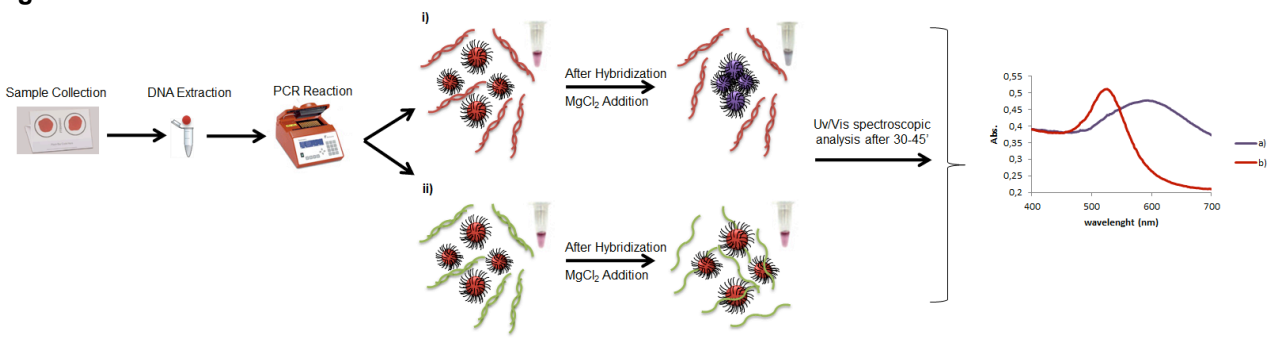

Figure 1 – Blood sample was collected from finger prick using a lancet and stored in FTA™ Indicated Micro Card (Whatman, UK). DNA extraction was carried out for subsequently PCR reactions. After PCR reaction, Au-nanoprobe was mixed with PCR products and a hybridization step was carried out i) after salt addition in the presence of a non-complementary or unrelated PCR a colorimetric change is visible

from red to purple after a certain time, ii) after salt addition in the presence of a fully complementary PCR product no colorimetric change is visible and the solution remains red. This color observation can also be observed by UV/Vis spectroscopy.