Genetic Testing for Determination of Multiple Single Nucleotide Polymorphism (SNP) Phase Information

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Background

As humans have two copies of each gene, multiple mutations in different loci may be found on the same strand of DNA (cis for the same strand and trans for different strands). The placement of these single nucleotide polymorphisms (SNPs) is called “SNP phasing”. Phase information, i.e. whether SNPs occur cis or trans to each other, is important because some mutations can mask the deleterious effects of others when they occur cis to each other. For example, thrombophilia is associated with two mutation sites in the methylenetetrahydrofolate reductase (MTHFR) gene. However, only when these two mutations occur trans to each other is the diseased phenotype observed. Despite the importance of SNP phasing, this information is often missed when using routine DNA sequencing technologies, as they randomly fragment the DNA before sequencing. Currently, if phasing is deemed necessary, the DNA of both parents has to be sequenced in addition to the patient’s. Alternatively, additional long-range sequencing tools have to be used. However, the widespread use of the aforementioned strategies is limited by labour-intensive protocols and the need for a high-power computing device for data analysis.

Technology Overview

This invention provides a novel strategy for resolving the phase information of multiple SNP sites based on nucleic acid self-assembly processes. This consists of a two-step oligonucleotide probe-based hybridisation assay, in which the first step is a toehold-mediated strand displacement reaction to interrogate the presence of the two SNPs. A second reaction dependent on the first is then conducted to differentiate cis from trans SNPs. The proposed strategy could serve as a good complement to primary DNA sequencing data in lieu of an additional resequencing step, which would be less efficient and more costly. A diagnostic tool capable of simply and quickly resolving the exact allelic content of chromosomal copies remains elusive. This invention provides a conditional hybridisation assay platform using short DNA probes for SNP phasing. All of the existing techniques and patents covering SNP phase detection require the use of either bioinformatics software, single-molecule sequencing methods or a microarray of a combination of primers. These are all expensive and laborious multi-step techniques. The present invention is a direct oligonucleotide hybridisation-based assay designed to phase SNPs only via fluorescence detection.

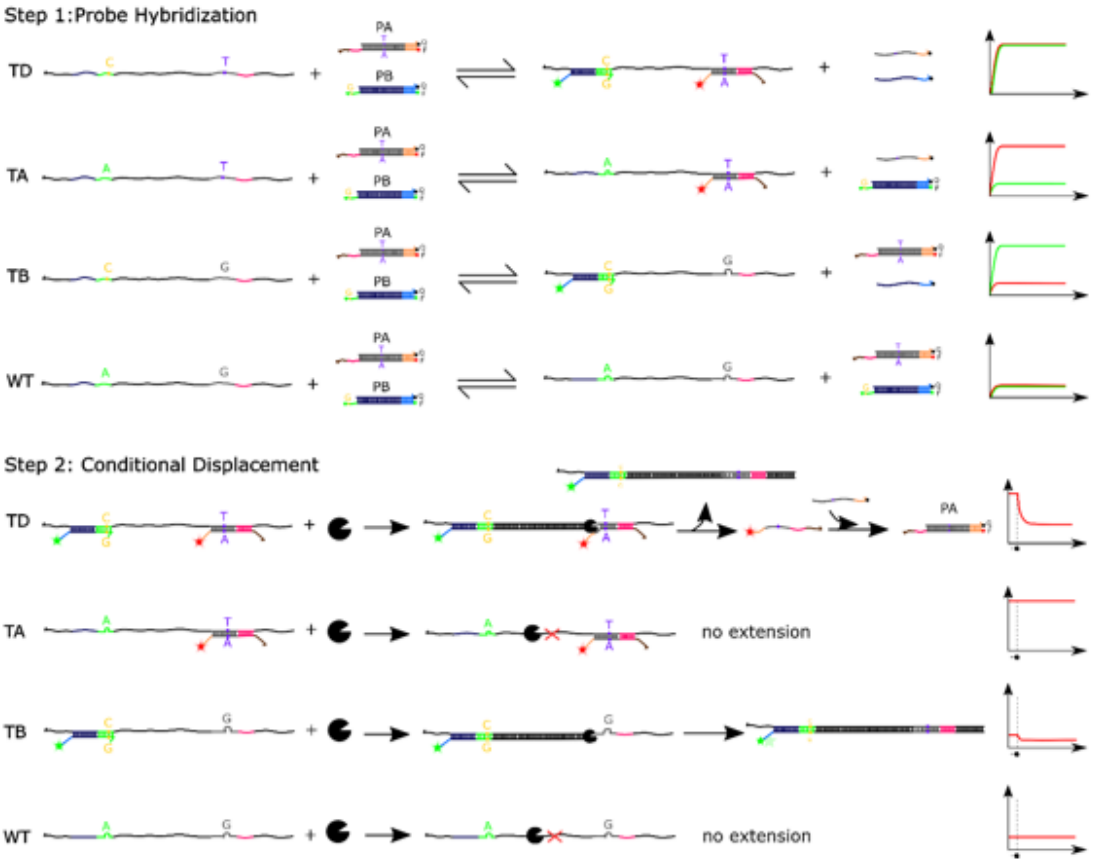
Applications

- Diagnostic kits that incorporate SNP phasing

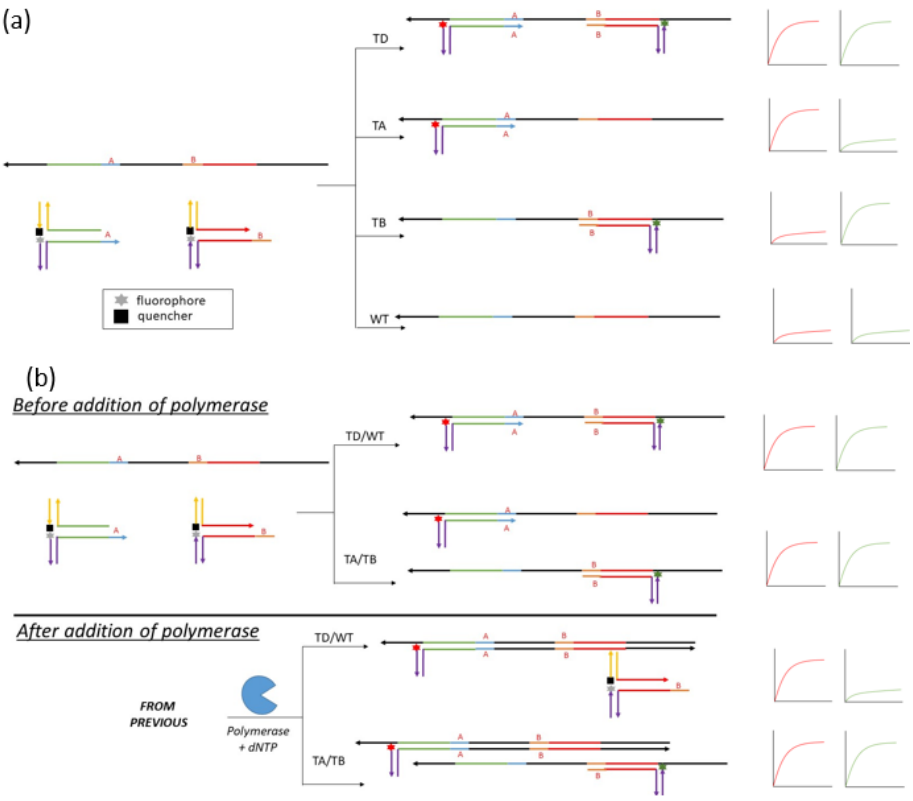
Patents

- PCT Patent no.: PCT/CN2018/078938
- US Patent Pending: -
- China Patent Pending: -

Figures



(A) The two double-stranded probes bind to the corresponding SNP site when present, and a hypothetical fluorescence readout is shown for each target case. (B) The second step involves the addition of a polymerase, and the corresponding fluorescence signals are also shown (TD: target with two SNPs; TA: target with SNP A; TB: target with SNP B; WT: wild type).



(A) Products of the X-probe and corresponding targets with the expected time-course fluorescence signal profile for the two fluorophores. (B) Demonstrates the possibility of differentiating TD/WT and TA/TB diplotypes on the addition of polymerase through the fluorescence readout (TD: target with two SNPs; TA: target with SNP A; TB: target with SNP B; WT: wild type).