Single nucleotide polymorphisms detected by temperature-modulated high-performance liquid chromatography

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Single nucleotide polymorphisms (SNPs) are new generation markers. An open question in research on SNPs is how to find novel SNPs in a natural population? To this end, we developed an approach of high-throughput genotyping by applying the temperature-modulated heteroduplex analysis (TMHA) based upon ion-pair reversed-phase high-performance liquid chromatography. This approach allowed a very efficient resolution of identically sized PCR products with a single nucleotide change. This approach is also known as denaturing high-performance liquid chromatography (DHPLC). However, the terms of both TMHA and DHPLC should be considered carefully. Firstly, it is DNA molecules that are denatured in high-performance liquid chromatography but not the method or the instrument. Secondly, the temperature in the liquid chromatograph is modulated according to Tm (melting temperature) of the DNA fragments. As is known, Tm is the temperature at which a population of double-stranded nucleic acid molecules becomes half-dissociated into single strands, and under this temperature, half of the DNA molecules are still not denatured. Thirdly, the targets for detection are both the homoduplexes and the mismatched heteroduplexes, which are double-stranded nucleic acid molecules and are annealed. The term DHPLC may lead to confusion in annealing and denaturing. In addition, since TMHA can be carried out using gel electrophoresis and HPLC, there is a need to distinguish HPLC from gel electrophoresis. Therefore, we suggest a new term for this technology, which is TmHPLC (temperature-modulated high-performance liquid chromatography). This term not only provides a clear definition for this new method, but also includes both TMHA and HPLC. More importantly, TmHPLC implies that the temperature in HPLC is modulated according to Tm of the DNA fragments. Our results show that this approach leads to significant reduction of sequencing efforts when searching for novel SNPs. This implies that TmHPLC is a sensitive, accurate and cost effective approach to screening sequence variation in human genome.

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PII: S0531-5131(02)00840-3