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METHOD

A High-Throughput Mutation Detection Method Based on Heteroduplex Analysis Using Graft Copolymer Matrixes: Application to Brca1 and Brca2 Analysis

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Abstract: We present here a new approach to electrophoretic heteroduplex analysis (EHDA) based on improved matrixes.

EHDA is an appealing technique for the detection of unknown point mutations because of its simplicity and high throughput. We present here a new matrix for electrophoretic heteroduplex analysis much more sensitive for insertions, deletions, and substitutions than reported for previous EHDA separations and also superior to DHPLC. This separation matrix is based on a copolymer with a comb architecture, poly(acrylamide-*g*-polydimethylacrylamide), made of a high molecular weight polyacrylamide backbone grafted with poly(dimethylacrylamide) side chains. The effect of operational parameters on electrophoretic resolution and sensitivity to singlenucleotide mismatches was studied using a collection of samples from patients bearing mutations in the breast cancer predisposition genes *BRCA1* and *BRCA2*. Seventeen fragments (10 mutations), implying mostly substitutions on fragments with sizes ranging from 200 to 600 bp, were analyzed using a single set of separation conditions. A success rate of 94% was achieved with a qualitative analysis in terms of number of peaks, and 100% identification of mutations was obtained with a more quantitative test using peak width analysis. This strong improvement of performance with regard to previous HAD methods is attributed to a composite mechanism of separation, combining steric and chromatographic effects. It opens the route to a significant reduction of development time and operation cost for diagnostic and genomic applications.