Parallel detection of HNF-1α mutations on PNA zip-code microarray by single base extension reaction

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In the present study, we exploited the superior features of peptide nucleic acids (PNAs) to develop an efficient PNA zip-code microarray for the detection of hepatocyte nuclear factor-1 (HNF-1α) mutations that cause type 3 maturity onset diabetes of the young (MODY). A multi-epoxy linker compound was synthesized and used to achieve an efficient covalent linking of amine-modified PNA to an aminated glass surface. PCR was performed to amplify the genomic regions containing the mutation sites. The PCR products were then employed as templates in a subsequent multiplex single base extension reaction using chimeric primers with 3' complementarity to the specific mutation site and 5' complementarity to the respective PNA zip-code sequence on the microarray. The primers were extended by a single base at each corresponding mutation site in the presence of biotin-labeled ddNTPs, and the products were hybridized to the PNA microarray. Compared to the corresponding DNA, the PNA zip-code sequence showed a much higher duplex specificity for the complementary DNA sequence. The PNA zip-code microarray was finally stained with streptavidin-R-phycoerythrin to generate a fluorescent signal. Using this strategy, we were able to correctly diagnose several mutation sites in exon 2 of HNF-1α with a wild-type and mutant samples including a MODY3 patient. This work represents one of the few successful applications of PNA in DNA chip technology.