

Primer extension technique for the detection of single nucleotide in genomic DNA

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For diagnosis of many genetic disorders where the nature of the DNA alteration is known, it is quite enough to determine which nucleotide (normal or substituted) is present in certain site of the gene. I describe here simple and fast technique for detection of single nucleotide in certain position of genomic DNA which may be adopted to any genetic disease with known mutations. This method is based on the repeatedly one nucleotide extension of an oligonucleotide complementary to the genomic sequence adjacent to the nucleotide to be determined. Fig. 1 illustrates this technique on the model experiment.

30-mer and 20-mer oligonucleotides complementary to the known sequence in human genome near the 'cystic fibrosis gene' (1) were used to show the ability of this method to detect correctly single nucleotide. A 50 μ l reaction mix contained 1 μ g of human genomic DNA, and 1 pM of 30-mer or 20-mer oligonucleotide in PCR buffer [16.6 mM (NH₄)₂SO₄, 67 mM Tris (pH 8.8), 6.7 mM MgCl₂, 10 mM 2-mercaptoethanol, 6.7 mM EDTA, 170 mg/ml BSA]. Four identical reaction mixtures were prepared for each primer. Only one of four (α -³²P)dNTPs (Amersham, 3000 Ci/mmol) was added to each reaction mixture (50 μ Ci per mix).

Samples were heated at 98°C for 7 min and cooled to 68°C for 2 min. Primer extension was initiated by addition of 1 unit of Taq-polymerase with incubation for 2 min at 68°C. Subsequent cycles were carried out at 92°C for 1 min and 68°C for 1 min. 20 μ l of samples after 30 cycles of primer extension were electrophoresed in 20% PAG containing 8 M urea for 2 h at 50 v/cm. Gel was exposed to X-film for 2 h. As it may be seen from Fig. 2, 30-mer oligonucleotide was converted into ³²P-labelled 31-mer oligonucleotide only with [α -³²P]dGTP, 20-mer oligonucleotide was converted into 21-mer ³²P-labelled oligonucleotide with [α -³²P]dCTP only. This is in agreement with known sequence of the corresponding region of genome (1), where C is adjacent to 3'-end of the sequence identical to 20-mer oligonucleotide, whereas G is downstream 3'-end of the sequence identical to the 30-mer oligonucleotide (Fig. 1).

REFERENCE

1. Williams, C. *et al.* (1988) *Lancet* ii, 102-103.

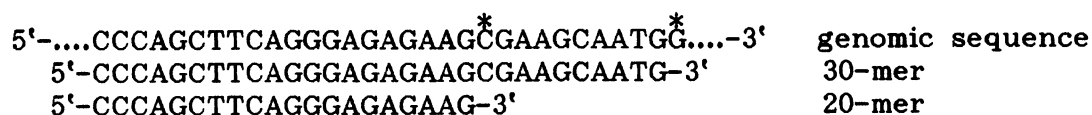


Fig. 1.

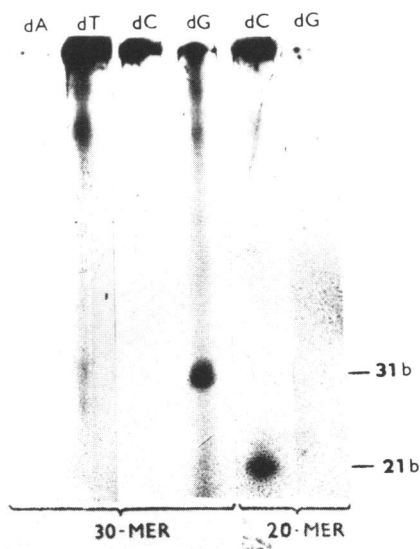


Fig. 2.