DIGOXIGENINE LABELED SEQUENCING OF THE HPRT GENE

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Protocols for radioactive sequencing of the Hypoxanthine-guanine-phosphoribosyltransferase(HPRT) gene have been reported by different authors. In contrast, only few described non-radioactive techniques using expensive sequencing automats. After having established a radioactive technique (modified according to Gibbs et al. 1990) we wanted to establish also a non-radioactive method.

Methods:

DNA extraction and PCR reactions

Genomic DNA was isolated from peripheral blood according to a non-organic method (Miller et al. 1988). The nine exons of the HPRT gene were amplified symmetrically in single PCR reactions (modified according to Gibbs et al. 1990) and amplified assymmetrically in second PCR reactions. The products of each PCR reaction were purified using glassmilk (Geneclean, Bio 101, La Jolly, USA).

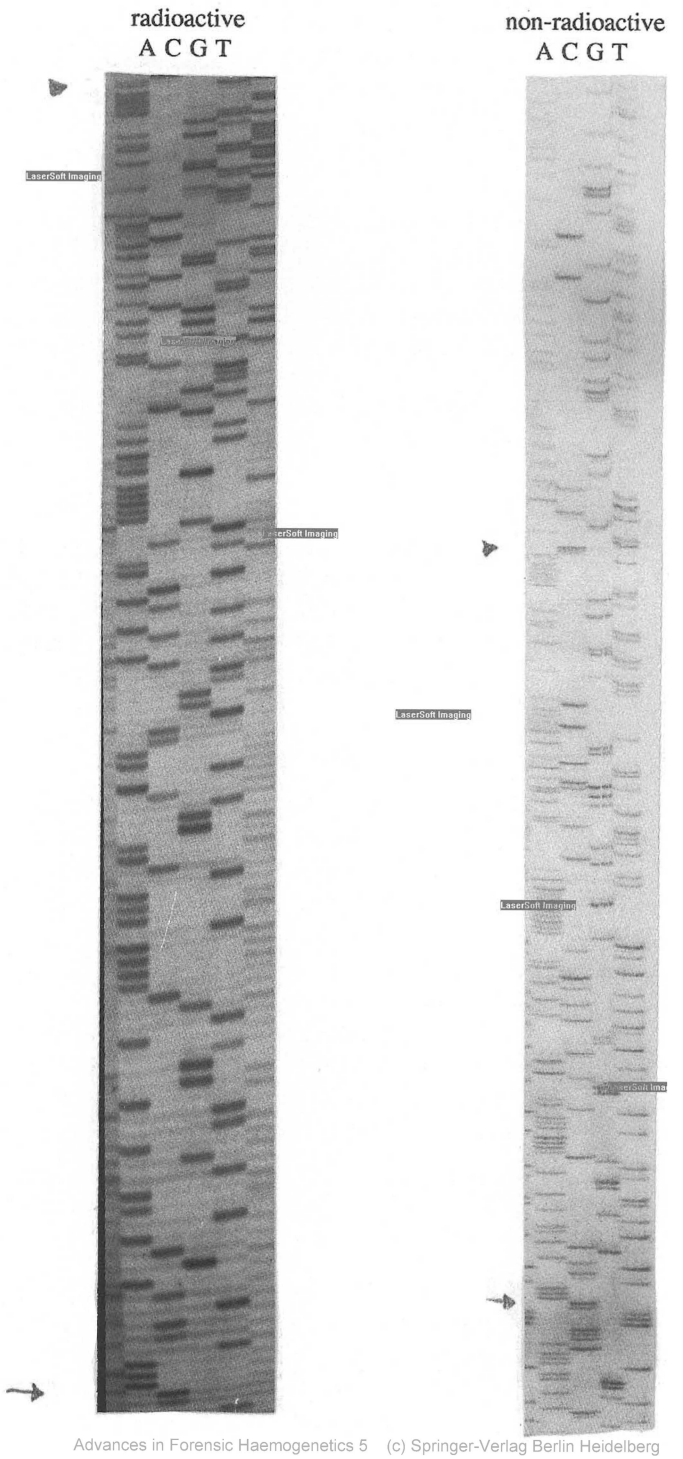
Sequencing reactions

Sequencing reactions were performed according to the method of Sanger et al. (Sequenase 2.0 Kit, USB, Bad Homburg). In contrast to the radio-active method, were ^{35}S dATP was incorporated during labeling, digoxigenine-marked primer were used in the non-radioactive technique. Sequencing electrophoresis was carried out in a direct blotting electrophoresis apparatus (GATC, Konstanz) using a 4% polyacrylamide urea gel in TBE buffer. The sequencing products were blotted onto a nylon membrane which was transported past the lower end of the sequencing gel. The sequencing products were crosslinked to the membrane by UV light (transluminator, Pharmacia-LKB, Freiburg). Visualization was carried out with digoxigenine antibody conjugated alkaline phosphatase (DIG Nucleic Detection Kit, Boehringer Mannheim).

Results:

The results of sequencing reactions of exon 2 are shown in figure 1.
In the non-radioactive technique about 200 to 300 bp were readable, compared to 100 to 200 bp in the radioactive technique in one application on the gel. Sequencing gels could be run multiply in the direct blotting apparatus with comparable results. Sequencing reactions tended to become weaker within months, due to loss of digoxigenine on the primer.

Figure 1: Comparison of radioactive and non-radioactive direct sequencing of exon 2 of the HPRT gene.
Arrows indicate the same region within the sequence.



Discussion

Non-radioactive sequencing with digoxigenine marked primer was comparable to the radioactive technique. Advantages may be: 1. longer parts are readable in one application. 2. multiple use of sequencing gels. 3. easy waste disposal. As a disadvantage digoxigenine labeled sequencing is still a sensible technique, which may be improved in the future by incorporation of digoxigenin labeled nucleotides in the sequencing reaction.

References:

Gibbs RA, Nguyen P-N, Edwards A et al.: Mutliplex DNA detection and exon sequencing of the hypoxanthine phosphoribosyltransferase gene in Lesch-Nyhan families. Genomics 7, 235-244 (1990)

Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acid Res 16, 1215 (1988)

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