Diagnosis of mutations by the PCR double RFLP method (PCR-dRFLP)

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The diagnosis of mutations that are responsible for human genetic diseases is important, and several molecular biology techniques based on polymerase chain reaction (PCR) (1), have been specially developed for this purpose (2—9). For some of these techniques it is the presence or the absence of a PCR product that constitutes the analytical step, i.e., proves the presence of the mutation. In order to discriminate between wild-type and mutant alleles, the analytical PCR requires specific and adapted PCR conditions for each mutation studied (concentration of each PCR primers set, temperature of hybridization, concentration of MgCl₂). Consequently, conditions must be continually adapted for the study of several different mutations. However, when a mutation creates or destroys a natural or an artificial (5—8) restriction site then PCR-RFLP is usually used to diagnose the mutation despite all other available diagnostic techniques. However, PCR-RFLP presents a technical difficulty since a defective restriction enzyme activity can be confused with the loss of the restriction site. Thus, it has been recommended that PCR products are digested in the presence of an excess of restriction enzyme for a long period of time (8). Despite these precautions, the presence of a restriction site is experimentally easier to ascertain than its absence. In order to overcome this difficulty, we have developed a method which we call the PCR double RFLP (PCR-dRFLP). For each studied DNA, two modified nested PCRs are performed at the same time. One pair of PCR primers is designed to introduce a restriction site specific for the wild-type allele while the second pair of primers is designed to introduce a restriction site specific for the mutant allele. Each PCR product is then analyzed by RFLP. These two RFLP allow an unambiguous interpretation of the results.

A summary of the method used is presented in Figure 1. Nested primers were selected so that the nested PCR gives an amplification product of approximately 90 bp for all mutations studied. Nested primers used to construct restriction sites border the site of the mutation. The interpretation of results of PCR-dRFLP is then always dependent upon the presence of a restriction product that has an expected size of approximately 70 bp. The Wp and Mp PCR products are 90 bp long before digestion and are cleaved to 70 and 20 bp fragments upon digestion with the appropriate restriction enzyme. By convention, for each studied DNA, the Wp product digested by the enzyme ErW is displayed in lane w and the Mp product digested by the enzyme ErM is displayed in lane m of the gels. Cleavage of Wp...
A sequence where a GG on the wild-type allele is replaced by a C on the mutant allele is given as follows: (GG / C). Sequences of primers are in upper case letters; mismatched bases are in bold characters; lower case letters are sequences following the primer on the genomic DNA and are given to locate the restriction site (underlined). Restriction enzymes ErW and ErM are described for each mutation.

![Figure 2. Examples of 17 genotypes establish by PCR-dRFLP.](image)

For the eight mutations studied in this report, the PCR-dRFLP system gave unambiguous results. Compared to the numerous techniques previously described, PCR-dRFLP possesses several advantages; the technique is simple and very robust, it does not necessitate precise quantification of the studied DNA, and although nested PCR is used, there is no direct manipulation of the first PCR product. The interpretation of results is supported by the presence of either one or two restriction sites as a function of the homozygous or heterozygous nature of the DNA being investigated. As a consequence, the two results obtained for a DNA allow the totally reliable diagnosis of the genotype of the sample studied.

Primers sequences and specific software used in this work are available on request.

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**REFERENCES**