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Rapid insertional mutagenesis of DNA by polymerase chain reaction (PCR)

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The polymerase chain reaction (PCR)(1) has become a powerful tool for site directed mutagenesis (2). Here we describe a very rapid procedure to introduce DNA segments into sequences devoid of suitable cloning sites at the primer binding sites. Two subsequent PCR steps are required. In the first step (Fig. 1A) the sequence between the primer pr1 and primer pr2 is amplified (Fig. 1B lane 1). The mutagenic primer pr1 consists of the sequence to be inserted flanked by short sequences complementary to the template. In a second PCR (Fig. 1A) the amplified fragment itself without further purification is used as a primer in combination with primer pr3 (Fig. 1B lane 3). In the second PCR a fragment containing the two restriction endonuclease sites (X and Y in Fig. 1A) is amplified. These are used to replace the unmutated part of the template by the amplified fragment including the insertion. We have applied this method to introduce the 36 bases leader sequence of alfalfa mosaic virus RNA4 into the genome of a wheat dwarf virus gene replacement vector. Since no purification of the amplification products is required mutant clones (10-30% of total analyzed) were achieved in four days.

The PCR conditions were as follows: PCR I was carried out in a reaction volume of total 50µl containing 50mM KCl, 10mM Tris-HCl pH8.3, 1.5mM $MgCl_2$, 300uM dNTP each, 0.01% gelatin, 2U Taq polymerase (Perkin Elmer Cetus), 40pg template DNA, 100pM primer each. PCR II was the same as PCR I instead 5µl (300ng of amplified fragment estimated from agarose gel) assay volume of PCR I was directly used for priming. Denaturation: 2 min 92° C; annealing: 5 sec RT; synthesis: 3 min 68° C with 30 cycles for each step. The primers were synthesized on a Applied Biosystems Model 380B DNA synthesizer.

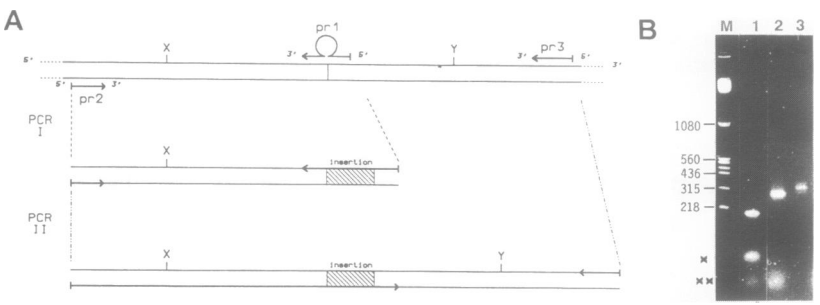

Figure legend: Figure 1A: Scheme for mutagenesis procedure, Figure 1B: EtBr stained 2% agarose gel; (M) size marker in bp; (1) product of PCR I; (2) for comparison PCR with primers 2 and 3 only; (3) product of PCR II; *: primer 1, **: primer 2 or primer 2 and 3 respectively.

References:

(1) Saiki et al. (1985) Science 230:1350-1354.

(2) Higuchi et al. (1988) Nucleic Acids Res. 16:7351-7367. Vallette et al. (1989) Nucleic Acids Res. 17:723- 1989. Kadowaki et al. (1989) Gene 76:161-166.

