A simple and rapid method for generating a deletion by PCR

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Site-directed mutagenesis including deletions, insertions and base-substitutions is one of the strongest tools for examining the biological or biochemical properties of cloned genes or gene control elements. Application of this technique has been, however, often limited because of the absence of a suitable restriction site or the complexity of procedures for generating a desired mutation. We report here a simple and rapid mutational method by using polymerase chain reaction (PCR) (1), in which oligonucleotide primers are designed in inverted tail-to-tail directions to amplify the cloning vector together with the target sequence (Fig. 1). A deletion is generated by amplification with primers that have a corresponding gap between their 5′ ends. After the PCR with these primers, amplified linear DNA is self-ligated, and used to transform appropriate competent cells.

We use human papillomavirus (HPV) type 16 E7 open reading frame cloned into pGEMEX™-1 (Promega) as a template. A pair of primers, 5′-TTAAATGACAGCTCAGAGGA-3′ and 5′-TGTCTCTGGTTGCAAATCTA-3′, was prepared for deleting HPV 16 nucleotide position 619−642. PCR of 25 cycles at 94°C, 2 min, 55°C, 2 min and 72°C, 4 min was done in 100 μl of reaction mixture containing 20 mM Tris-HCl, pH 8.8 at 25°C, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.1% Triton X-100, 100 μg/ml acetylated BSA, 200 μM each of dNTPs, 2 ng of the template plasmid, 2 μM each of the primers and 2 units of Vent™ DNA polymerase (New England Biolabs). Use of Vent™ DNA polymerase was required for simplifying the protocols because *Taq* DNA polymerase adds one nontemplate-directed nucleotide to its products and generates 3′ overhanging ends (2), whereas Vent™ DNA polymerase possesses 3′−5′ exonuclease activity to yield blunt ends, which are ready for self-ligation (3). A portion of agarose gel-purified PCR product was self-ligated in 10 μl of 66 mM Tris-HCl, pH 7.4, 8 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP, 5 units of T4 polynucleotide kinase and 5 units of T4 DNA ligase at 14°C for 12 h, followed by use for transformation of JM109. Sequencing of about 300 base pairs each of 16 resulting plasmids shows excellent efficiency of 100% with only one misincorporation (Figure 2). We note that our inverted amplification method will be available for generating an insertion or a base-substitution.

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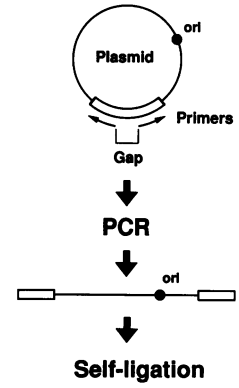

Figure 1. Strategy for generating a deletion in a sequence cloned into a plasmid. Solid lines, open boxes and small arrows indicate plasmid DNAs, target sequences and PCR primers, respectively. PCR in inverted directions is done with primers that have a gap between their 5′ ends, and the resulting deleted DNA is self-ligated to transform *E. coli*.

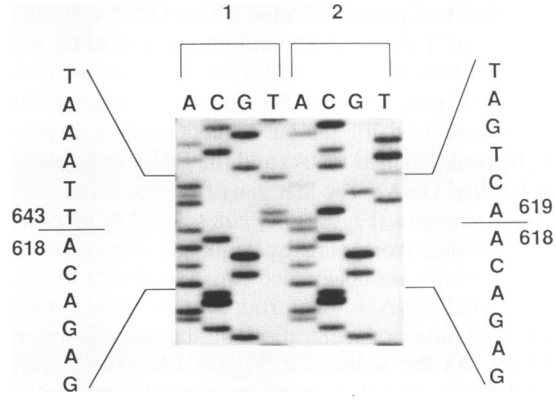

Figure 2. Sequencing analysis of a deletion generated in HPV 16 E7 open reading frame. The deleted (lanes 1) and original (lanes 2) E7 sequence were examined by dideoxy chain termination method. Numbers beside the nucleotide codes indicate nucleotide positions of HPV 16.