Volume 17 Number 7 1989 Nucleic Acids Research

Directed mutagenesis using PCR

Laurent Dulau, Alain Cheyrou and Michel Aigle

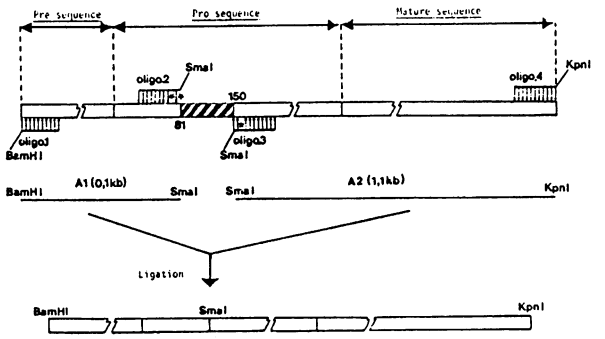
Laboratoire de Génetique, Université de Bordeaux II, Avenue des Facultés, 33405 Talence cedex, France
Submitted January 26, 1989

In order to study the determinism of the protease A (PEP4 vacuolar gene (1) product of Saccharomyces cerevisiae), the following modifications were introducing in PEP4. First the coding sequence of the gene was delimited by two unique restriction sites allowing insertion in varied cloning multisite polylinkers. Secondly the Pro sequence was modified by deleting nucleotides 81 to 150 and creation of a SmaI restriction site. We realised these steps by amplification (2) of two fragments which delimit the deletion. The primers contained the new restriction sites BamHI, KpnI and SmaI (see figure). The two amplified fragments were separatly cloned in M13 phage RF DNA. Sequencing (3) of these clones (A1: 0.1 kbp and A2: 1.1 kbp) confirmed the presence of the three point mutations and the deletion. No accessory mutations were observed and reconstitution of the modified gene was achieved (see figure). So, P.C.R can be used as a powerfull and rapid technic for gene modification.

Amplification procedure: in two 100 µl separate reaction mixtures (16.6 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 10 mM β mercapto-ethanol , 65 mM Tris-HCl pH 9.0 , 200 µg/ml BSA, 5% DMSO, 200 µM dNTP, 50 pmole of each primer), 0.5 ng of PEP4 cloned gene in an yeast multicopy plasmid (kindly provided by P.Neuville) were amplified. To amplify the A1 fragment we used two primers 1, 2 and one unit of TaqI polymerase (Biolabs). For A2 fragment amplification, primer 3, 4 and 7.5 units of TaqI Pol. were used. The conditions of reaction were:

- for A1: Denaturation; 30 seconds 95°C, Hybridization; 30 seconds 55°C, Synthesis; 1 min. 70°C
- for A2: Denaturation; 1 min. 95°C, Hybridization; 1 min. 55°C, Synthesis; 10 min. 70°C

After 35 cycles, a termination step: 20 min. 70°C, was perfomed.



Sequences of the oligonucleotide primers
1: 5'-GGATCCATGTTCAGCTTGAAAGCATTATTG-3'
2: 5'-CCCGGGCTTGTGGACTTTTGCAGC-3'
3: 5'-CCCGGGCAAAAGTACTTGACTCAA-3'
4: 5'-GGTACCAAGGATAGGGCGGAGAAGTAAGAA-3'
Underlined nucleotides are not complementary with the template sequence.

*represent a mis-match between a base of the template sequence and a base of the oligonucleotide primer.

Reference: 1) Woolford, C.A., Daniels, L.B., Park, F.J., Jones, E.W., VAN Arsdell, J.N. and Innis, M.A. (1986) Mol. Cell. Biol., Vol.6, N°.7, 2500-2510. 2) Saiki, K.R., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.E. (1988) Science, Vol.239, 487-491. 3) Sanger, F., Niecklen, S., and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463.