Nucleic Acids Research

Using the polymerase chain reaction to modify expression plasmids for epitope mapping

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We have used the polymerase chain reaction (PCR) (1) to create deletions within existing expression plasmids and have used these modified plasmids to refine our epitope map of SV40 large T antigen. Our procedure is similar to that of Vallette *et al.* (2) but has the advantage that the modified region is not excised from the plasmid. We perform PCR around the entire plasmid from primers whose 5' ends define the region to be deleted. Self-ligation of the PCR product recircularises the plasmid. If the region to be deleted contains a unique restriction site, host plasmid can be cut at this site after the ligation reaction to provide a strong positive selection for recombinants.

We linearised a pUC19 construct (below) with BamH1, performed PCR using the primers shown, self-ligated the product, cut again with BamH1 and used the product to transform *E.coli.* We performed DNA sequencing to check clones identified by colony blotting which were positive with antibodies already mapped to the retained region and negative with antibodies mapped to the deleted region (3). The recombinant was then used to map PAb416 to T antigen amino acids 1-106.

Uncut plasmid can also be amplified. To keep N terminal deletions in frame it may be better to use primers containing restriction sites and perform sticky end rather than blunt end ligation because we have found an additional A/T base pair at the ligation site with several pairs of primers, despite kinasing the PCR product before ligation. We recommend sequencing the subcloned plasmid in the region of interest.
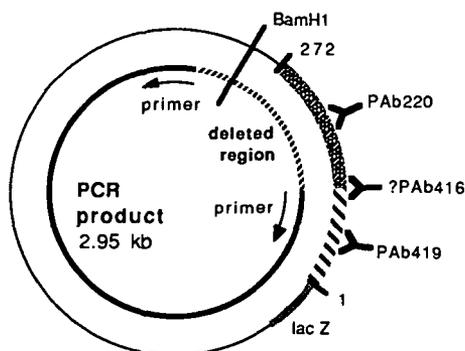

Diagram of pUC19 construct expressing T antigen amino acids 1-272. PCR was performed with Cetus Taq polymerase according to the manufacturer's instructions using 21mers for 30 cycles of 92°C for 1 min, 50°C for 1 sec, 72°C for 5 min. PAb220 maps within the deleted region (▩▩▩ amino acids 107-272), PAb419 maps within the retained region (⦚⦚ amino acids 1-106).

References: 1. Saiki, R.K., Scharf, S., Falloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A. and Arnheim, N. (1985) Science **230**, 1350-1354. 2. Vallette, F., Mege, E., Reiss, A. and Adesnik, M. (1989) Nucl. Acids Res. **17**, 723-733. 3. Mole, S.E., Gannon, J.V., Ford, M.J. and Lane D.P. (1987) Phil. Trans. R. Soc. Lond. B **317**, 455-469; Mole, S.E. unpublished data.