Band-stab PCR: a simple technique for the purification of individual PCR products

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PCR amplification of DNA can give rise to non-specific products particularly when degenerate primers (2) are employed. The excision and purification of the band of interest from a gel is required to permit further characterisation of the product by direct DNA sequencing, digestion with restriction endonucleases, cloning, mutation detection by SSCP (3), or use as a probe.

We have developed a simple method for obtaining and further amplifying a specific single PCR product from a mixture of PCR products of different molecular weight.

Specific DNA fragments generated by combined PCR subtraction hybridization (1) and cloned in pUC18 were used as template for PCR. Each PCR reaction was performed in sterile 0.5 ml tubes using 100 μl final reaction volumes containing Tris, pH 8.3, 10 mM; KCl, 50 mM; $MgCl_2$, 1.5 mM; gelatin, 0.01% (w/v); dNTP's, 200 μM; primer (M13), 1 μM; 0.5 units of AmpliTaq DNA polymerase (Perkin Elmer Cetus). Evaporation within the tubes was prevented by the addition of a 100 μl mineral oil overlay. Mixtures were subjected to 25 cycles of amplification (2). Fifteen microliters of PCR product was loaded onto a 3% NuSieve agarose, 1% SeaKem agarose (w/v), (FMC BioProducts) composite mini-gel and electrophoresed for 1 h at 65 v. The gel was stained with ethidium bromide, rinsed in water and viewed on a UV Transilluminator (short wavelength). After removing excess water from the gel surface with Whatman 3 MM paper the appropriate band was stabbed with a hypodermic needle which was subsequently dipped with gentle agitation into a fresh PCR reaction mix to permit amplification and electrophoresis as above.

This method facilitated the isolation of a single PCR product from a mixture of other products of different molecular weight in less than 1 h (Figure 1). Drying the gel surface, the use of a fine needle and careful stabbing of the band were essential for specific product purification. The band-stab technique minimises disruption of the gel surface and avoids contamination with closely migrating ampliproducts which is often not possible by other methods. In addition, the band-stab method enables direct amplification of the band of interest, therefore obviating the requirement for the additional purification steps mentioned above. The method should be generally applicable but may be particularly useful in PCR protocols where non-specific ampliproducts arise, for example when employing degenerate primers or reduced primer annealing stringency when screening for novel sequences.

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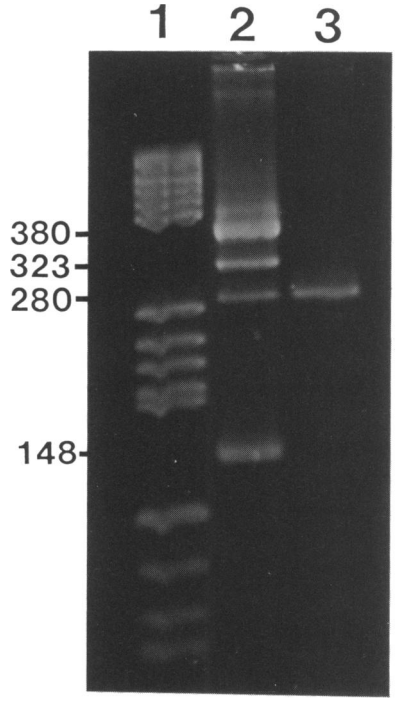

Figure 1. A mixture of DNA fragments generated by combined PCR subtraction hybridization (1) was cloned into pUC18. Four of the clones were mixed, amplified by PCR using M13 primers and 10 μl of product was electrophoresed (lane 2). The 280 Kb product was band stab-purified (lane 3) before electrophoresis. Lane 1 Molecular weight markers (pBR322 HaeIII digest).