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Rapid plasmid insert amplification with polymerase chain reaction

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A DNA probe used for prenatal diagnosis of cystic fibrosis, 5'met (1), cloned in vector pUC19, has been amplified with M13 sequencing primers by the polymerase chain reaction technique, PCR (2). The amplification provides high yield, pure probe which gives a signal with low background. The procedure is much quicker and easier than conventional plasmid preparation, digestion and insert fragment isolation.

We isolated plasmid DNA by alkaline extraction and PEG precipitation. Five ng of this DNA was subjected to PCR for 30 cycles in a total volume of 100 µl, with 3 units of Taq DNA polymerase (Cetus), 1 X Taq PCR buffer, 100 picomoles of each of the two primers (New England Biolabs sequencing primer # 1211 and reverse sequencing primer #1201), and 80 nanomoles of dNTP (dATP + dCTP + cGTP + dTTP, Boehringer Mannheim). The 100 µl mixture was covered by 30 µl of light mineral oil (Sigma). Plasmid DNA was denatured by heating to 95°C for 2 minutes followed by a 2 minute annealling of primers and plasmid at 50°C, and then incubation at 72°C for 2 minutes to extend synthesis of the insert.

The inserted copy was amplified by a factor of about 10^3 to 10^4 (1 ng to µg quantities). This is less than genomic copy amplification due to limitation of the yield by primer and dNTP concentrations and by enzyme activity. The insert DNA was isolated by spermine precipitation and resuspended in TE^{-4}. One fourth of this was labeled by the random primer technique with ^{32}P- dCTP and used as a probe.

This procedure can be applied to any insert cloned in a vector with available flanking sequencing primers. The only limitation is the length of insert, but recent reports indicate success with up to 2kb.

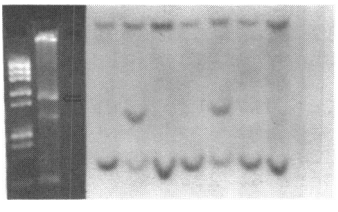

Figure: Amplified insert (left, arrow) and hybridized blot (right)

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