Generation of DNA probes for peptides with highly degenerate codons using mixed primer PCR

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The use of the polymerase chain reaction (PCR, (1)) is well suited for the generation of DNA probes from a peptide sequence. The limited specificity of each primer is multiplied when 2 are used in combination. We have used 2 primers, each a mixture of 128 species, to amplify genomic DNA coding for amino acids 1 - 25 of diabetes associated peptide (DAP (2,3)). The primer sequences represented all codon choices for amino acids 1 - 5 and 20 - 25 plus an Eco RI linker. After 30 cycles, a 20 ul aliquot was run on a gel and a prominent band of the predicted size was seen (Fig.1). About half the Eco RI clones from this band encoded DAP 1-25 though many primers were used :-

AAA TGT AAC ACC GCC ACA TGT GCA ACG CAG CGC CTG GCA
AAT TTT TTA GTT CAT TCC AGC AAC AAT TTT GGT GCC AT

Although mixed primer PCR clearly works under a wide range of conditions (4) the degree of specific amplification depends critically on the use of sufficient primer and choice of annealing temperature. When, as here, exon structure can be predicted and site of synthesis is unclear, genomic DNA should be used rather than cDNA. Either way, the use of mixed primer PCR is an extremely powerful general technique for obtaining probes from genomic DNA when only limited amino acid sequence is available.

A 100 ul reaction contained 1 ug of human genomic DNA (5), 40 ug of each primer mix and 4 U of Taq DNA polymerase in the buffer recommended by Cetus. Each cycle was 2 min at 92°C, 48°C and 70°C. Track A - unreacted, tracks B & C - annealed at 40°C and 48°C respectively, track M - size markers.

References.

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