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Highly degenerate, inosine-containing primers specifically amplify rare cDNA using the polymerase chain reaction

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Highly degenerate oligonucleotide primers constructed from corresponding amino acid sequence are shown to be useful in the polymerase chain reaction (PCR). Unmixed oligonucleotide primers A and C were designed to amplify a 698 bp fragment encoding the voltage-sensitive sodium channel in rat brain (1). Eco RI sites flanked the primer sequences to facilitate subcloning of the fragment. Lane 2 reveals that the fragment of predicted length was amplified. High stringency hybridization (lane 4) of a ^{32}P-labeled Na^+ channel cDNA probe (nt 3361-5863, (1)) to the amplified fragment following Southern transfer indicated that the fragment represents the sodium channel. Mixed oligonucleotides B and D, targeted to the same regions as A and C, were synthesized such that each potential degeneracy within the genetic code was dealt with by mixing nucleotide bases and inserting inosine where degeneracy was maximal. Primer B is capable of recognizing 576 different target sequences assuming inosine is neutral with regard to base pairing. Primer D has 1024 potential target sequences. PCR amplification using primers B and D (lane 3) and subsequent Southern analysis (lane 5) were conducted under the same conditions with identical results, indicating that the degenerate primers work as well as the defined ones.

To date, PCR primers described in the literature have had either complete (2) or slightly degenerate (3) complementarity to the targeted DNA sequence. The use of highly degenerate oligonucleotides containing inosine greatly expands the use of PCR; amino acid sequences involving residues encoded by multiple codons can be used to design oligonucleotide primers. Mixed, inosine-containing primers have been successfully used by this laboratory to amplify other cDNA fragments. However, the specificity of this approach varies greatly depending on the cDNA concentration, the length and sequence of the primers, and the parameters of the PCR reaction. Therefore, some adjustment is necessary with each application.

5′ Primers	3′ Primers
A: GGAATTCC GGTGTCATCATAGACAAC	C: GGAATTCC AACAGGCAGATCATGCTGTT
B: GGAATTCC GGIGTIATCATCGACAACT (T T T T; A A)	D: GGAATTCC AAIAGGCAIATCATIGTGTT (CA; C A)

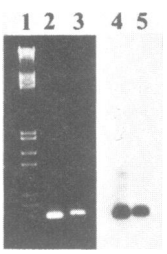

Figure legend. Fragments amplified using primers A and C (lane 2) or primers B and D (lane 3) were analyzed by agarose gel electrophoresis, transferred to nitro-cellulose and hybridized (50% formamide, 5X SSPE at 42°C) to a ^{32}P-labeled Na^+ channel cDNA (lanes 4 and 5, respectively). Lane 1 contains Eco RI/Hind III digested lambda phage DNA. The Taq polymerase based PCR reactions (100 µl) were run as recommended by Cetus Corp. using 50 ng of first strand rat brain cDNA. Twenty µl were applied to the gel. The reaction parameters were: denaturation for 1 minute at 94°C, primer annealing at 37°C for 2 minutes, and extension at 72°C for 2 minutes. Thirty cycles were run using the Perkin Elmer Cetus DNA Thermal Cycler.

References

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