Enhanced evolutionary PCR using oligonucleotides with inosine at the 3′-terminus

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The polymerase chain reaction (PCR) is a powerful tool for the enzymatic amplification of individual DNA sequences from unique oligonucleotide primers and a small amount of target DNA (1). In order for a successful PCR to occur each of the oligonucleotide primers must hybridize to sequences flanking the gene of interest. Mismatched nucleotides at the 3′-ultimate and 3′-penultimate positions have previously been shown to be detrimental to the amplification process (2). This is primarily due to a need for a perfect 3′ base-pair to allow enzymatic synthesis rather than any thermodynamic effect on duplex formation. Mismatch of the oligonucleotide primers is particularly important in evolutionary PCR since the oligonucleotide primer pair is usually based upon nucleotide sequence information from one organism which may have diverged from other species of interest many millions of years ago. Previously, deoxyinosine has been successfully substituted into degenerate positions of PCR primers for ambiguous nucleotide positions (3). We have chosen to substitute deoxyinosine at the 3′-ultimate nucleotide position in order to determine whether or not this substitution is compatible with DNA synthesis using *Taq* DNA polymerase. This facilitates PCR at neutral loci in different primate species where the 3′ terminal nucleotide may have mutated.

Oligonucleotide primers complementary to the unique DNA sequences flanking a recently inserted human-specific (HS) Alu family member were synthesized with and without inosine at the 3′-ultimate nucleotide position. The sequence of the primer pair, optimal annealing temperature (55°C), and source DNA cell lines were previously reported (4). The primers were used to amplify orthologous loci within human, chimpanzee, gorilla, orangutan, green monkey (old world monkey), and owl monkey (new world monkey) genomes. Amplification results in the production of a 450 bp band if an Alu family member is present at a particular locus (Figure 1, human DNA amplified with and without primers containing inosine). A 150 bp band (Figure 1, all non-human primate DNAs except green monkey amplified using non-inosine primers) is amplified if no Alu family member is present at a particular locus, or both bands are amplified if the locus is heterozygous for the Alu insertion (none shown). Figure 1 shows the amplification of the HS C4N4 Alu insertion locus using primers with and without inosine. These data show that the inclusion of inosine at the 3′ position resulted in the amplification of a 150 bp product (no Alu family member present) from green monkey DNA, which was not amplified using otherwise identical primers which did not terminate with inosine. The lack of amplification of green monkey DNA using primers without inosine is probably the result of a mutation at the terminal nucleotide position within one of the oligonucleotide primers. This is not surprising since the human/old world monkey divergence is thought to have occurred 25 million years ago. In several other cases we have found the incorporation of a 3′ inosine in the PCR primers to increase the reliability and consistency of the PCR amplification in other species. These data suggest that the inclusion of inosine at the 3′-terminal nucleotide position is advisable for evolutionary PCR.

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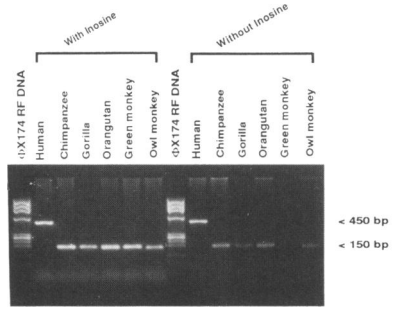

Figure 1. PCR analysis of an individual Alu family member at orthologous positions within primate genomes. PCR amplification was carried out in a reaction mixture which contained 2.5 units of *Taq* DNA polymerase (BIOS Corporation), 200 μM dNTP's, 750 ng of each unique primer, 100 ng of target DNA from the species of interest and a 10×reaction buffer for 30 cycles as previously described (4). One fifth (20 μl) of the reaction products were fractionated on a 2% agarose gel with 0.5 μg/ml ethidium bromide and visualized directly by UV fluorescence. The marker was ΦX174 RF DNA digested with *Hae*III.