SYNTHETIC OLIGONUCLEOTIDES: PROBLEMS AND FRONTIERS OF PRACTICAL APPLICATION

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The use of synthetic oligonucleotide primers for amplification of a variable number of tandem repeats loci for paternity testing

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At present, mainly two methods based on Southern blot analysis are used for paternity testing and forensic identification: hybridization with cloned DNA from some VNTR loci and phage M13 DNA. Using unique oligonucleotide primers flanking the variable repeated sequences we have applied the PCR technique with DNA polymerase Tth from Thermus thermophilis for amplification of 4 different VNTR regions: 3'- flanking region of apoB gene, locus D17S30, 3'- flanking region of IL-6 gene and locus D1S58 in human chromosomes 2, 17, 7 and 1 respectively. The allele numbers are from 4 to 21 and the sizes of amplified fragments from 170 to 900 bp. Ethidium-bromide stained agarose and polyacrylamide gels were used for detection of amplified polymorphic DNA fragments. The alleles of used loci are inherited in an autosomal codominant manner. We have analyzed the distribution of polymorphic DNA fragments in unrelated individuals and families. The use of these types of VNTR gives a total probability of identity between two non-related persons of about $1-2x10^{7}$. The typing based on amplification of VNTR loci can be routinely achieved within 2 - 3 days compared to 1 - 2 weeks for classical DNA "fingerprints".

The uses of synthetic oligonucleotide primers for prenatal diagnosis of sex in chorionic villi via the polymerase chain reaction

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The precise determination of the sex of foetus is a matter of interest in prenatal diagnosis of inherited diseases. We have developed a simple and rapid test for sex diagnosis based on detection of Y and X chromosomal DNA by PCR technique with DNA polymerase Tth from Thermus thermophilis. We have used synthetic oligonucleotide primers for amplification of the fragments from 3 repeated sequences in Y and X chromosomes. Primers Y1 and Y2 encompass 149 bp of a 3.4 kb repeat sequence from the heterochromatic region of the long arm of the Y chromosome, but have an increased risk of an incorrect diagnosis of sex because of its inability to detect specific sequences in individuals lacking this region of the Y chromosome. The only repetitive DNA family likely to exhibit the properties of significant chromosome specificity is the alphoid satellite family located in pericentromeric regions of all human chromosomes. Primers Y3, Y4 flank the 170 bp fragment of the alphoid repeats of the Y chromosome and primers X1, X2 flank the 130 bp fragment of alphoid repeats of the X chromosome. We have used all these primers for sex diagnosis on a series of 11 samples of chorionic villi: 7 from female foetuses, 4 from male foetuses. The sex of foetuses were controlled by cytogenetic analysis of chorionic villus culture.