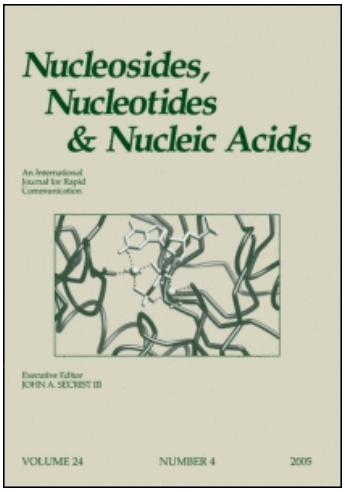

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Convenient Procedure for Oligonucleotide-Directed in vitro Mutagenesis of Cloned DNA

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CONVENIENT PROCEDURE FOR OLIGONUCLEOTIDE-DIRECTED *in vitro* MUTAGENESIS OF CLONED DNA

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Summary. A new modification of the oligonucleotide-mediated mutagenesis technique has been used to produce specific base changes in the double-stranded plasmid DNA.

A procedure has been designed for changing specific nucleotides in a DNA sequence with high efficiency. The method involves using the special constructed cloning vectors pHS1 and pHS2. These plasmids are derivatives of pBR322 in which the EcoRI-HindIII region has been replaced by synthetic duplexes carrying SmaI, HpaI and XhoI sites, in addition to EcoRI and HindIII sites. The DNA fragment to be mutagenized is cloned in pHS1 (or pHS2) using restriction sites situated close to the SmaI and HpaI sites. The recombinant plasmid obtained is digested with one of these enzymes to produce double-stranded DNA with blunt ends. This linear DNA is a substrate for exonuclease III. The digestion in controlled conditions produces duplex with protruding single-stranded 5'-regions which include the site of the desired mutation. The synthesis of DNA by DNA-polymerase I (Klenow fragment), primed in part by the synthetic oligodeoxyribonucleotide containing the desired mutation, leads to linear heteroduplex. The closed circular heteroduplex is formed by ligation. After transformation into E.coli, DNA replication generates homoduplexes, some of which contain the mutation. Colony hybridization with the same ^{32}P-labeled oligonucleotide is used to select mutants. The yield of the mutants in 10-20%. This technique can be extended to replicative form of M13 vectors. It can be also applied to any DNA suquence which has a unique site of restriction endonuclease generating blunt ends.

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