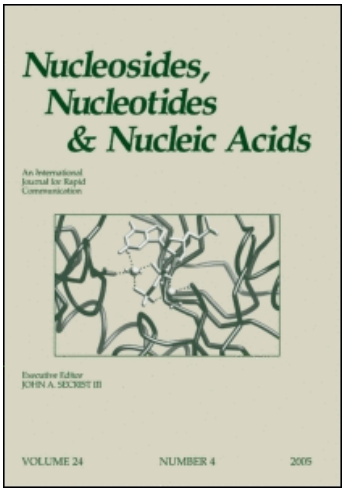

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A Rapid Protocol For Site-directed Mutagenesis and Gene Synthesis Using Synthetic Oligonucleotides

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A RAPID PROTOCOL FOR SITE-DIRECTED MUTAGENESIS AND GENE SYNTHESIS USING SYNTHETIC OLIGONUCLEOTIDES.

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Summary : DNA segments of 100 to 150 nucleotides in length have been assembled by overlapping synthetic oligonucleotides and cloned directly into the single stranded vector M13 without purification of the synthetic DNA segments prior to cloning.

Blocks of synthetic oligonucleotides were designed in a way to harbour sticky ends at both the 5' and the 3' ends of the synthetic DNA segment to be generated. Cloning was done into the polylinker region of an M13 vector which had been cut with the appropriate restriction endonucleases to generate the corresponding sticky ends, and phosphatase treated. Up to 15 different overlapping synthetic oligonucleotides were mixed together with the M13 vector DNA, hybridized and ligated. In the ligation reaction only those synthetic DNA molecules generated by the overlapping oligonucleotides which contain both the appropriate 5' and 3' sticky ends will recircularize the vector DNA and give plaques after transformation of competent E.coli (JM107). Thus, we took advantage of the high specific biological selection for synthetic DNA molecules containing both sticky ends for recircularization of the vector DNA, instead of going through several rounds of hybridization, ligation and gel purification of the synthetic DNA fragments. M13 phage DNA from individual plaques was prepared and the correct sequence of the synthetic DNA insert was confirmed by DNA sequencing using the dideoxy sequencing technique.

Using this protocol different synthetic enhancer sequences have been assembled. Furthermore various enhancer mutants were constructed simply by exchanging the appropriate oligonucleotides on both strands making this technique a powerful tool for site-directed mutagenesis.

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