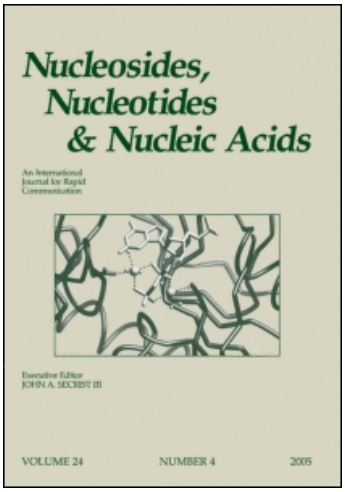

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Versatile Methodology for the Construction of Artificial Genes

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VERSATILE METHODOLOGY FOR THE CONSTRUCTION OF ARTIFICIAL GENES

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Abstract. The chemical-enzymatic synthesis of several genes has been carried out with the use of a versatile approach to the assembling long DNA duplexes.

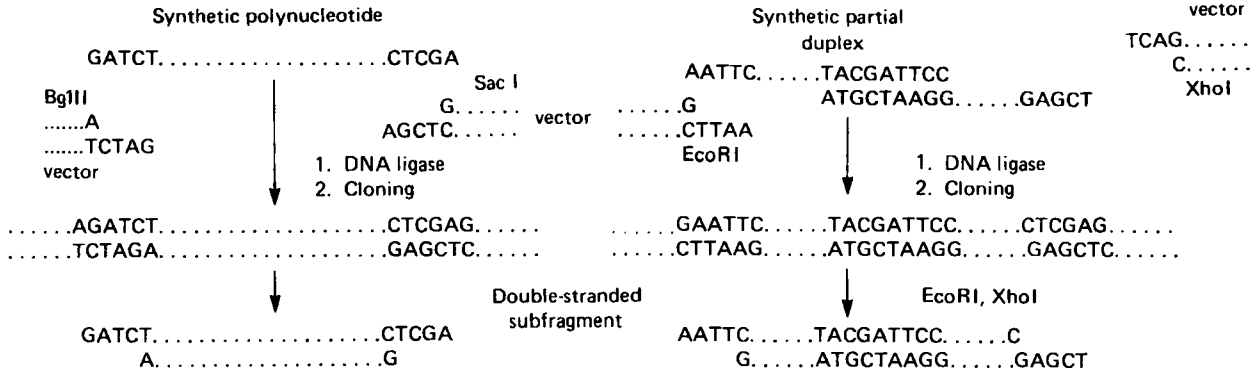
In the course of investigations on the chemical-enzymatic synthesis, cloning and expression of DNA fragments, a versatile methodology for assembling double-stranded polynucleotides,which permits preparation of synthetic DNAs of practically unlimited lengths, have been worked out in our laboratory [1,2]. This methodology is based on the DNA construction from preliminary cloned subfragments and the use of temporary restriction sites for cloning individual subfragments and assembling the target polynucleotide.The places for temporary restriction sites can be chosen arbitrarily during experiment planning, and they form the frontiers of the modules from which the gene will be finally assembled. Such supplementary sites may be inserted at any place of DNA sequence containing a pair of self-complementary nucleotides: AT, TA, CG or GC. To achieve the insertion , the sequence of a gene is as if pushed apart and a tetra- or pentanucleotide is incorporated between the selected pair of nucleotides to supplement the fragment up to the sequence of the desired restriction site. Thus, to construct an EcoRI restriction site a tetranucleotide (AATT) had to be inserted between the nucleotide residues of the GC pair in both DNA strands.

This methodology is particularly applicable in conjunction with plasmids (or phages) containing polylinker region.

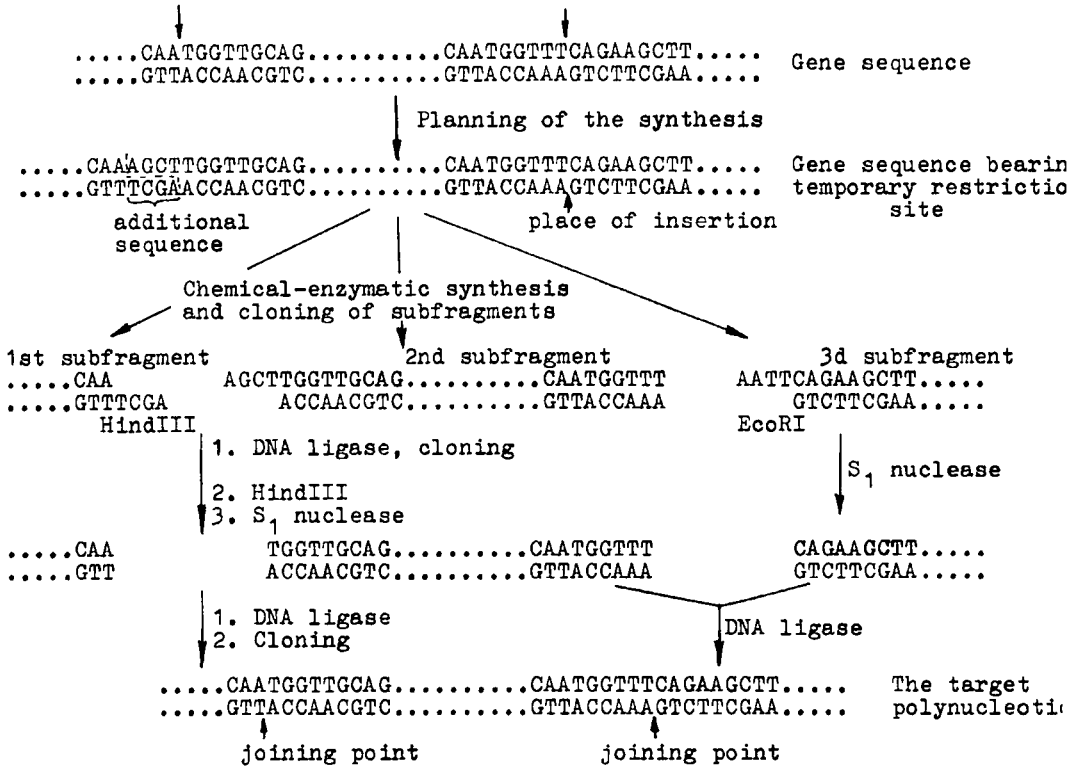
Two such plasmid vectors, pPLE1 and pPLE2, containing 14 unique sites for restriction endonucleases, have been constructed by us earlier [2,3].

The subfragments required for the synthesis of the target gene could be prepared by any of the known methods: by ligation of chemically produced oligonucleotides [4], or by in vitro repair synthesis of partial duplexes formed by oligonucleotides possessing stretches of complementary sequences at their 3'-ends [5], or by combination of these methods. For better results, before joining the duplexes had to be purified by cloning. In order to minimize the synthetic work, these procedures can be simplified. Thus, it is possible to synthesize chemically and clone only one of the chains of the target DNA duplex. This single-stranded polynucleotide is inserted into a vector molecule between two restriction sites, one of them having 5'-protruding end and the other having 3'-protruding end, and after ligation cloned without repairing in vitro. If both restriction sites chosen for the subfragment cloning have only 5'-(or 3'-)protruding ends, the partial duplex formed by two long oligonucleotides having stretches of complementary sequences at their 3'-(or 5'-)ends can be used with the same perpose and cloned without repairing in vitro also. These procedures shown in Scheme 1 was used in our laboratory in the syntheses of a λ p_L promoter region and its mutants and also in the syntheses of fragments of a gene for M_r 22,000 zein from maize endosperm[6].

Synthetic gene can be assembled from subfragments by two different routes (Scheme 2). One route involves successive cloning of the DNA subfragments in a suitable cloning vehicle, so that finally a complete gene which carries in its sequence all the inserted restriction sites can be assembled in the vector. To remove these temporary sites, the plasmid DNA can be successively digested by corresponding restriction endonucleases, treated by S_1 nuclease and blunt-end ligated. Temporary sites can be also removed by oligonucleotide-directed mutagenesis [1]. This pattern of



SCHEME 1



SCHEME 2

DNA assembling is particularly advantageous when aiming at production of several vertions of a gene, that can be achieved by replacement of certain subfragments of a gene by modified sequences encoding mutant proteins. Another assembling route involves consecutive joining of subfragments with simultaneous removal of temporary restriction sites. In this case, immediately after ligation at the joining point of two subfragments a sequence corresponding to the exact structure of a gene is formed.With this route it does not matter which nucleotide pair residues is at the blunt-ends of subfragments connected; so that division of a target gene sequence into subfragments can be effected at any place, and not only between self-complementary nucleotide pairs (Scheme 2). The use of synthetic oligonuc-

leotides as specific hybridization probes is effective for determination of correctness of module joining in the assembled molecule. The effectiveness of the above described methods was demonstrated in our laboratory on the syntheses of several functionally active DNAs, such as a gene coding 198-231 segment of bacteriorhodopsin [7], a gene coding for human preproinsulin,and these methods are used now for the construction of a gene for maize zein protein.

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