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A cloning cartridge of λ t_o terminator

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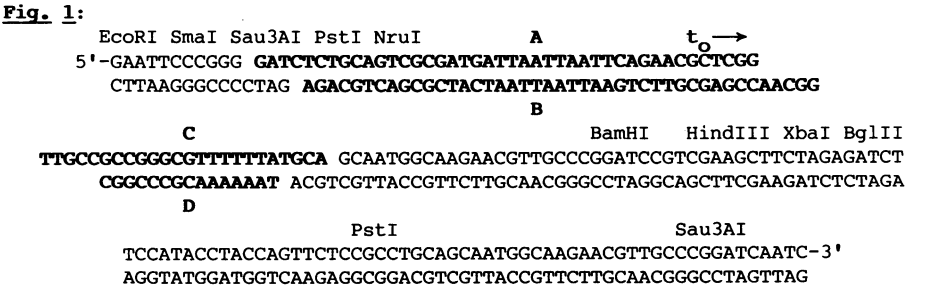
The t_o terminator of bacteriophage λ is one of the strongest terminators known. Therefore it should be an efficient terminator of overexpressed gene transcripts starting from strong promotors such as the λ promotors P_L and P_R or the E. coli hybrid promotor tac. Here we present a cartridge of the t_o terminator cloned into a high copy number run away plasmid.

Initially we synthesized the entire plus and minus strand of the t_o terminator. The formation of stable secondary structures in both strands prevented an efficient purification of the products. To avoid this problem we divided the synthesis into four parts (Fig. 1, oligos A to D). After purification and deprotection the oligonucleotides B and C were 5'-phosphorylated, hybridized to A and D, and ligated to form the complete double-stranded terminator sequence.

The run away plasmid pHC624[1] was cleaved with HindIII and SalI, filled in with MMLV polymerase, and blunt-end ligated to yield pSS1 (1999 bp). To remove the PstI site in the Amp^r gene the BglI/PvuI fragment of pSS1 was replaced by the same fragment from pUR292[2] yielding pSS3. The terminator fragment was cloned into BglII/PstI digested pSS3 to yield a plasmid with t_o adjacent to the multiple cloning site of pHC624 (pSS18, 2033 bp). A 364 bp fragment including t_o was isolated from pSS18 after digestion with HaeIII and BglII. This fragment was digested with Sau3AI and the desired 88mer including the terminator was inserted into BamHI cleaved pSS3 under reformation of a unique BamH1 site (pSS9, 2087 bp). The DNA sequence from the EcoRI site to the BglII site of pSS9 (Fig. 1) was verified by Maxam-Gilbert sequencing. pSS9 is available upon request. We thank J. Ott for synthesizing the oligonucleotides.

Fig. 1:



References: 1. Boros I., Posfai, G. & Venetianer, P. (1984) Gene 30, 257-260;
2. Rüther, U. & Müller-Hill, B. (1983) EMBO J. 2, 1791-1794.

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