Cloning of synthetic oligodeoxynucleotides may result in high frequency promoter mutations in *E.coli*

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We have chemically synthesized variants of the *leuV* promoter from *E. coli*. Promoters were created by synthesis of two complementary oligodeoxynucleotides using a Biosearch Cyclone DNA synthesizer. The two strands were purified by electrophoresis on 8 percent polyacrylamide, 7 M urea gels, annealed, and ligated into plasmid pSL100 (1). Transformants were selected for chloramphenicol resistance expressed from the *leuV* promoter. DNA sequence analysis of cloned promoters revealed a group of point mutations clustered between -18 and +8 relative to the transcription start point (Figure 1). Approximately 54 percent of all clones examined contained at least one base change, with most containing just one. The mutations reported here occur at a frequency (0.7 per nucleotide) similar to that observed by other workers (2,3).

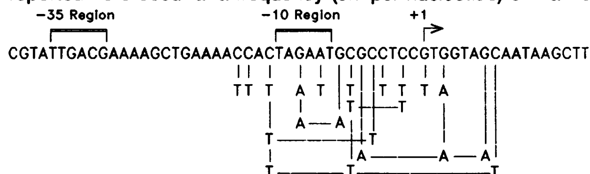

Figure 1. Top line shows wild type *leuV* promoter. Second line represents single base changes (10/28 clones sequenced). Subsequent lines represent multiple base changes (5/28 clones).

A model for mutagenic modifications occurring during chemical synthesis of oligodeoxynucleotides was recently proposed (2). Briefly, a reagent involved in capping steps (N,N-dimethylaminopyridine) forms a fluorescent adduct at the O-6 position of guanine. Oligos carrying this modification fluoresce when illuminated with long wave ultraviolet light (366 nm). Upon treatment of the oligo with ammonium hydroxide, residues carrying this adduct can be converted to 2,6-diaminopurine deoxyribonucleoside, a naturally occurring analogue of adenine which is able to pair with deoxythymidine. Subsequent cloning and replication of the DNA would result in G to A transitions.

The data presented here support this model. Ninety five percent of the substitutions represent either G to A or C to T transitions. It should be noted that these are probably equivalent events occurring on opposite strands. No corresponding A/T to G/C transitions have been observed. Oligos used to generate these clones were observed to fluoresce when illuminated with UV light (366 nm). A less aggressive capping reagent (N-methyl imadazole) has been suggested to prevent this problem (2). Oligos synthesized using this chemistry have not yielded any mutations and UV induced fluorescence has also been eliminated.

The most striking aspect of the mutations generated here is the high degree of clustering observed. Interestingly, the mutated region corresponds closely to the sequences thought to be single stranded during initiation of transcription (4). The *leuV* promoter initiates transcription at a high rate (1) and therefore may contain single stranded regions much of the time. This single stranded character may lead to a higher frequency of uncorrected errors in replication.

The mutations reported here represent a valuable collection for the study of promoter structure and function. However, these results indicate that extreme care should be excercised in the characterization of any cloned synthetic oligonucleotides. Further characterization of the promoters described is under way.

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