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Variants in clones of gene-machine-synthesized oligodeoxynucleotides

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We have found numerous sequence variants in clones of synthetic oligodeoxynucleotides containing tRNA genes. In four constructs, the frequency of variants was 8/20 or 40% per clone and 10/3152 or 0.3% per nucleotide (see table). Other researchers have also observed variants in synthetic oligos; thus, care must be exercised with this technology.

For each construct, two complementary oligos were synthesized at the UW Biotechnology Center using a machine (model 380A) and chemicals obtained from Applied Biosystems. O-methyl-phosphorous protection was employed. The oligos (purified by electrophoresis on a 12% polyacrylamide, 7M urea gel) were annealed and ligated into vector mp8 or a plasmid related to pEMBL. After transformation, clones were screened by hybridization with one of the oligos for the presence of the insert. Single-stranded DNA was prepared from positive candidates and sequenced by the dideoxy method. Each sequence was read independently a minimum of four times by two individuals using a computer program with a proofreading feature.

NAME	OLIGO LENGTH	NUMBER NORMAL	CLONES VARIANT	BASE CHANGE (POSITION)*	VARIANT/TOTAL BASES
EMBL-1	63 & 72†	3	1	G to A (74)	1/540
EMBL-2	78 & 87††	1	3	G to A (57) T to G (12) G to C (35)	3/660
EMBL-3	78 & 86	2	2	T to ' (7) T,T to ',' (13,50)	3/656
mp8-1	77 & 85	6	2	G to A (76) G,G to A,A (23,57)	3/1296

* Position from 5' end of longer oligo. ' is a base deletion.
† Insertion/deletion mismatch at position 51 of 72-long oligo.
††Insertion/deletion mismatch at position 21 of 87-long oligo.

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