***In vitro* methylation doubles the efficiency of oligonucleotide-directed mutagenesis**Renata D.Horton⁺ and Susan T.Lord*

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Directed mutagenesis using oligonucleotides as primers is an efficient method to produce mutations in DNA fragments (1,2). We have found that *in vitro* methylation of the primed heteroduplex DNA using *dam* methylase approximately doubles the yield of mutant plaques. Based on the results of Pukkila *et al.*, (3) we feel that *in vitro* methylation of the newly synthesized DNA blocks mismatch repair and thus increases the yield of mutant phage. We used a mutating primer complementary to M13mp18 with an A to T substitution which produces an in-frame amber stop codon in the lac Z segment. When phage were grown in JM101 in the presence of BCIG, plaques arising from replication of the mutated sequence were light blue or colorless while wild type plaques were blue. We sequenced several plaques to verify our phenotypic identification and to demonstrate that the appropriate mutation was made. Representative data are presented in Table I.

REACTIONS	WITHOUT METHYLATION			WITH METHYLATION		
	BLUE	WHITE	% MUTANTS	BLUE	WHITE	% MUTANTS
1. MU-O ALONE	166	21	11	316	106	25
	162	18	10	173	40	19
	150	24	14	309	161	34
2. MU-O + A	871	158	15	355	136	28
	347	91	21	288	141	33
	272	51	16	225	119	35
3. MU-O + B	110	17	13	305	99	25
	329	43	12	208	94	31
	122	20	14	231	92	29

TABLE I: Number of plaques counted. In each reaction 2.3 pmoles of mutating oligonucleotide (MU-O: CGACGCCAGTGGCAGCTTGC) was annealed to 0.12 pmoles of M13mp18, extended for 8 hours and ligated (2). The DNA was methylated with 100 μ M S-adenosylmethionine and 1 unit *dam* methylase (New England Biolabs) in 50 mM Tris-HCl, pH 7.4, 10 mM EDTA and 5 mM beta-mercaptoethanol at 37°C for 1 hr. Methylated DNA was diluted and transformed into *E. coli*. Reaction 2 included 2.4 pmoles of a second primer, oligo A (GCGCTTTTGGGGATCG) which hybridizes to mp18 at nucleotide #1396 and reaction 3 included 2.4 pmoles of oligo B (GAGCAAAAGAGATGATG) which hybridizes at nucleotide #4283.

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