

Altered Sites[®] II *in vitro* Mutagenesis Systems

By William H. Brondyk
Promega Corporation

Introduction

Promega's family of Altered Sites[®] Mutagenesis Systems features positive antibiotic selection in concert with the widely used approach of oligonucleotide-directed mutagenesis. The Altered Sites[®] technology uses oligonucleotides to restore inactive antibiotic resistance genes. The resulting antibiotic resistance produces selection for the mutant strand and, thereby, the desired mutation. The Altered Sites[®] II Systems use resistance/sensitivity to ampicillin and tetracycline; the Altered Sites[®] II Mammalian Systems use resistance/sensitivity to ampicillin and chloramphenicol. By activating one antibiotic gene (sensitive --> resistant) and simultaneously inactivating the second antibiotic gene (resistant --> sensitive), many rounds of mutagenesis are possible.

Altered Sites[®] II *in vitro* Mammalian Mutagenesis System

The Altered Sites[®] II *in vitro* Mammalian Mutagenesis System features the pALTER[®]-MAX Vector^(a,b), which allows for mutagenesis and mammalian expression from the same vector (1). [Figure 1](#) outlines the mutagenesis procedure, which can be performed using either single-stranded (ss) or double-stranded (ds) DNA templates. After cloning the gene of interest into the pALTER[®]-MAX Vector, a mutagenic oligonucleotide is hybridized to the template. The mismatch in the oligonucleotide is designed to produce the desired mutation. Following hybridization, high fidelity T4 DNA Polymerase extends the oligonucleotide to create a duplex structure. T4 DNA Ligase seals the nicks and the heteroduplex DNA then is transformed into a suitable *E. coli* host. Due to the antibiotic selection step using the Altered Sites[®] II technology, the yield of mutants can be as high as 90%. In one study that assessed 80 mutant clones, 71% were found to contain the correct base change and base deletions as directed by the Chloramphenicol Knockout Oligonucleotide. In a second study designed to assess the mutation rate of converting an *EcoR* I site from inactive to active, 15 of 16 clones (94%) contained the correct single base substitution.

^(a)U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

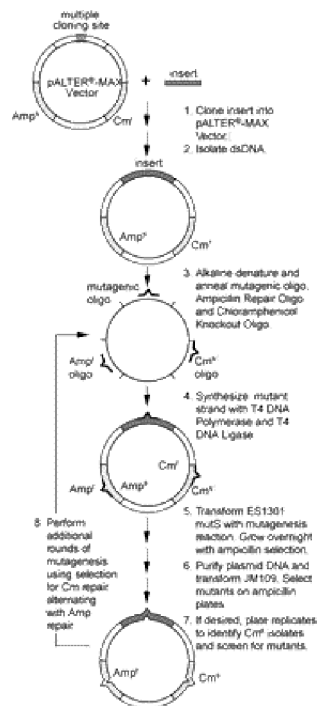


Figure 1. Schematic diagram of the Altered Sites[®] II Mammalian Mutagenesis System procedure.

The pALTER[®]-MAX expression vector simplifies cloning, mutagenesis and gene expression. The multiple cloning site of this vector is

nearly identical to that of other mammalian expression vectors available from Promega (pCI-neo^(a,b) (Cat.# E1841), pCI^(b) (Cat.# E1731) and pSI (Cat.# E1721) Vectors) providing ease in subcloning between different vectors. The T3 and T7 RNA Polymerase promoters flank the multiple cloning site and can be used to synthesize RNA from the sense and antisense strands of the cloned DNA insert. The CMV enhancer/promoter^(b) in concert with a chimeric intron drives strong constitutive expression of cloned genes in a variety of mammalian cell types. Also included is the SV40 late polyadenylation signal downstream of the multiple cloning site to facilitate the efficient processing of cloned DNA inserts lacking such signals. As evident in expression studies in CHO cells (Figure 2), the pALTER[®]-MAX Vector performs comparably to the pCI-neo Vector, which also contains the CMV promoter, chimeric intron and the SV40 polyadenylation signal. The pALTER[®]-MAX Vector also expresses chloramphenicol acetyltransferase (CAT) in both *E. coli*, where it serves as a selectable marker by conferring resistance to the antibiotic chloramphenicol, and mammalian cells, where it can serve as an internal control for transfection.

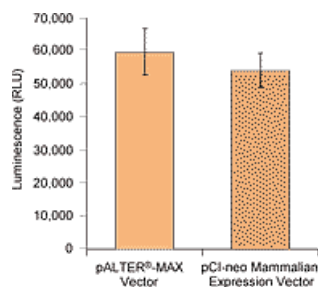


Figure 2. Comparison of expression levels of a cloned luciferase gene between Promega's pCI-neo Mammalian Expression Vector and the pALTER[®]-MAX Vector. Cells were seeded at 3×10^5 per 60mm dish in a 1:1 solution of F-12:DMEM + 10% fetal bovine serum medium, and were grown at 37°C in a 5% CO₂ atmosphere. The following day, the cells were transfected with 5µg of DNA per 60mm dish using the ProFection[®] Mammalian Transfection System -- Calcium Phosphate (Cat.# E1200). Cells were fed with medium at 24 hours, and harvested and assayed for luciferase expression 48 hours post-transfection using the Promega Luciferase Assay System^(c) as described (2). Expression levels were comparable in a CHO cell line. Similar results were obtained in COS7 and NIH 3T3 cell lines (data not shown).

^(b) The CMV Vector technology is the subject of U.S. Pat. No. 5,168,062 assigned to the University of Iowa Research Foundation.

^(c) U.S. Patent No. 5,283,179 has been issued to Promega Corporation for a firefly luciferase assay method which affords greater light output with improved kinetics as compared to the conventional assay.

Summary

The Altered Sites[®] II systems offer a number of advantages over other mutagenesis systems. Multiple mutations can be created in a single round of mutagenesis and multiple rounds of mutagenesis can be performed without additional subcloning steps. Furthermore, the Altered Sites[®] II technology does not require a unique restriction site within the vector, special strains to amplify the vector or ssDNA preparation.

References

1. *Altered Sites[®] II Mammalian Mutagenesis System Technical Manual #TM041*, Promega Corporation.
2. *Luciferase Assay System with Reporter Lysis Buffer Technical Bulletin, #TB161*, Promega Corporation.

Ordering Information

Product	Cat.#
Altered Sites [®] II <i>in vitro</i> Mutagenesis System	Q6210
Altered Sites [®] II <i>in vitro</i> Mutagenesis System and 1ml ES1301 <i>mutS</i> Competent Cells	Q6491
Altered Sites [®] II-Ex1 <i>in vitro</i> Mutagenesis System	Q6090
Altered Sites [®] II-Ex1 <i>in vitro</i> Mutagenesis System and 1ml ES1301 <i>mutS</i> Competent Cells	Q6501
Altered Sites [®] II-Ex2 <i>in vitro</i> Mutagenesis System	Q6080

Altered Sites [®] II-Ex2 <i>in vitro</i> Mutagenesis System and 1ml ES1301 <i>mutS</i> Competent Cells	Q6511
Altered Sites [®] II Mammalian <i>in vitro</i> Mutagenesis System	Q5590
Altered Sites [®] II Mammalian <i>in vitro</i> Mutagenesis System and 1ml ES1301 <i>mutS</i> Competent Cells	Q6600
pALTER [®] -MAX Vector (20µg)	Q5761
ES1301 <i>mutS</i> Competent Cells (1ml)	L1151

© 1997 Promega Corporation. All Rights Reserved.

Altered Sites, *pALTER* and *ProFection* are trademarks of Promega Corporation and are registered with the U.S. Patent and Trademark Office.