Site-directed mutagenesis of virtually any plasmid by eliminating a unique site.

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Abstract

We describe an efficient site-specific mutagenesis procedure that is effective with virtually any plasmid, requiring only that the target plasmid carry a unique, nonessential restriction site. The procedure employs two mutagenic oligonucleotide primers. One primer contains the desired mutation and the second contains a mutation in any unique, nonessential restriction site. The two primers are annealed to circular single-stranded DNA (produced by heating circular double-stranded DNA) and direct synthesis of a new second strand containing both primers. The resulting DNA is transformed into a mismatch repair defective (mut S) Escherichia coli strain, which increases the probability that the two mutations will cosegregate during the first round of DNA replication. Transformants are selected en masse in liquid medium containing an appropriate antibiotic and plasmid DNA is prepared, treated with the enzyme that recognizes the unique, nonessential restriction site, and retransformed into an appropriate host. Linearized parental molecules transform bacteria inefficiently. Plasmids with mutations in the unique restriction site are resistant to digestion, remain circular, and transform bacteria efficiently. By linking a selectable mutation in a unique restriction site to a nonselectable mutation, the latter can be recovered at frequencies of about 80%. Since most plasmids share common vector sequences, few primers, targeted to shared restriction sites, are needed for mutagenizing virtually any plasmid. The procedure employs simple procedures, common materials, and it can be performed in as little as 2 days.