Protocol : Mutant Strand Synthesis Reaction (Thermal Cycling)

Notes : Ensure that the plasmid DNA template is isolated from a dam+ E. coli strain. The majority of the commonly used E. coli strains are dam+. Plasmid DNA isolated from dam– strains (e.g. JM110 and SCS110) is not suitable. To maximize temperature-cycling performance, Stratagene strongly recommends using thin-walled tubes, which ensure ideal contact with the temperature cycler's heat blocks. The following protocols were optimized using thin-walled tubes.

1. Synthesize two complimentary oligonucleotides containing the desired mutation, flanked by unmodified nucleotide sequence. Purify these oligonucleotide primers prior to use in the following steps (see Mutagenic Primer Design).

2. Prepare the control reaction as indicated below:

5 µl of 10× reaction buffer
5 µl (25 ng) of pWhitescript 4.5-kb control plasmid (5 ng/µl)
1.25 µl (125 ng) of oligonucleotide control primer #1 [34-mer (100 ng/µl)]
1.25 µl (125 ng) of oligonucleotide control primer #2 [34-mer (100 ng/µl)]
1 µl of dNTP mix
1.5 µl of QuikSolution reagent
34 µl ddH2O (to bring the final reaction volume to 50 µl)
Then add: 1 µl of QuikChange® Lightning Enzyme

3. Prepare the sample reaction(s) as indicated below:
Note Stratagene recommends setting up a series of sample reactions using various amounts of dsDNA template ranging from 10 to 100 ng (e.g., 10, 25, 50, and 100 ng of dsDNA template) while keeping the primer concentration constant.

5 µl of 10× reaction buffer
X µl (10–100 ng) of dsDNA template
X µl (125 ng) of oligonucleotide primer #1
X µl (125 ng) of oligonucleotide primer #2
1 µl of dNTP mix
1.5 µl of QuikSolution reagent ddH2O to a final volume of 50 µl
Then add: 1 µl of QuikChange® Lightning Enzyme 8 QuikChange® Lightning Site-Directed Mutagenesis Kit

4. If the thermal cycler to be used does not have a hot-top assembly, overlay each reaction with ~30 µl of mineral oil.
5. Cycle each reaction using the cycling parameters outlined in Table I. (For the control reaction, use a 2.5-minute extension time.)

TABLE I
Cycling Parameters for the QuikChange Lightning Site-Directed

Mutagenesis Method

Segment	Cycles	Temperature	Time
1	1	95°C	2 minutes
2	18	95°C	20 seconds
		60°C	10 seconds
		68°C	30 seconds/kb of plasmid length*
3	1	68°C	5 minutes

* For example, a 5-kb plasmid requires 2.5 minutes per cycle at 68°C.