

# QuikChange™ Site-Directed Mutagenesis Kit

## INSTRUCTION MANUAL

Catalog #200518

Revision #108005h

### STORAGE CONDITIONS

**Epicurian Coli® XL1-Blue Supercompetent Cells: –80°C**

**All Other Components: –20°C**

**For in Vitro Use Only**

## LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Stratagene. Stratagene shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

## ORDERING INFORMATION AND TECHNICAL SERVICES

### United States and Canada

#### Stratagene Cloning Systems

11011 North Torrey Pines Road

La Jolla, CA 92037

**Telephone** (619) 535-5400

**Order Toll Free** (800) 424-5444

**Technical Services** (800) 894-1304

**Internet** techservices@stratagene.com

**World Wide Web** www.stratagene.com

### Stratagene European Contacts

Location	Telephone	Fax	Technical Services
Austria	660 312 526	660 312 527	017 956 7036
Belgium	0800 96078	0800 96024	027 13 12 11
Germany	0130 840 911	0130 762 088	0699 509 6197
Netherlands	0800 023 0446	0800 023 0447	0800 023 0448
Switzerland	0800 830 250	0800 825 225	01 800 9045
United Kingdom	0800 585 370	0800 783 0889	0171 365 1056

### Distributors

All other countries, please contact your local distributor (see *Stratagene Distributors* in this instruction manual for a complete listing).

# QuikChange™ Site-Directed Mutagenesis Kit

## CONTENTS

Introduction.....	1
Materials Provided.....	3
Additional Materials Required .....	3
Primer Design .....	4
Transformation Guidelines .....	5
Storage Conditions.....	5
Aliquoting Cells.....	5
Use of Falcon 2059 Polypropylene Tubes.....	5
Length of the Heat Pulse.....	5
Preparing the Agar Plates for Color Screening.....	5
Protocol.....	6
Setting Up the Reactions.....	6
Cycling the Reactions .....	7
Digesting the Products .....	7
Transforming into Epicurian Coli XL1-Blue Supercompetent Cells .....	8
Troubleshooting .....	10
Preparation of Media and Reagents.....	12
Related Stratagene Products .....	12
Stratagene Distributors .....	14
References.....	15
Endnotes .....	15
Quick-Reference Protocol .....	16

# QuikChange™ Site-Directed Mutagenesis Kit

Catalog #200518

## INTRODUCTION

In vitro site-directed mutagenesis is an invaluable technique for studying protein structure-function relationships and for identifying intra-molecular regions or amino acids, both of which may mediate gene expression and vector modification. Several approaches to this technique have been published, but these methods generally require single-stranded DNA (ssDNA) as the template<sup>1-4</sup> and are labor intensive or technically difficult. Stratagene's QuikChange™ site-directed mutagenesis kit\* allows site-specific mutation in virtually any double-stranded plasmid, thus eliminating the need for subcloning into M13-based bacteriophage vectors and for ssDNA rescue.<sup>5</sup> In addition, the QuikChange site-directed mutagenesis system requires no specialized vectors, unique restriction sites, or multiple transformations. This rapid four-step procedure generates mutants with greater than 80% efficiency. The protocol is simple and uses either miniprep plasmid DNA or cesium-chloride-purified DNA.

The QuikChange site-directed mutagenesis kit is used to make point mutations, switch amino acids, and delete or insert single or multiple amino acids. The QuikChange site-directed mutagenesis method is performed using PfuTurbo™ DNA polymerase<sup>‡,¶</sup> and a thermal temperature cycler.<sup>‡</sup> PfuTurbo DNA polymerase replicates both plasmid strands with high fidelity and without displacing the mutant oligonucleotide primers. The basic procedure utilizes a supercoiled double-stranded DNA (dsDNA) vector with an insert of interest and two synthetic oligonucleotide primers containing the desired mutation (see Figure 1). The oligonucleotide primers, each complementary to opposite strands of the vector, are extended during temperature cycling by using PfuTurbo DNA polymerase. Incorporation of the oligonucleotide primers generates a mutated plasmid containing staggered nicks. Following temperature cycling, the product is treated with Dpn I. The Dpn I endonuclease (target sequence: 5'-Gm6ATC-3') is specific for methylated and hemimethylated DNA and is used to digest the parental DNA template and to select for mutation-containing synthesized DNA.<sup>7</sup> DNA isolated from almost all *Escherichia coli* strains is *dam* methylated and therefore susceptible to Dpn I digestion. The nicked vector DNA incorporating the desired mutations is then transformed into Epicurian Coli® XL1-Blue supercompetent cells<sup>‡</sup>. The small amount of starting DNA template required to perform this method, the high fidelity of the PfuTurbo DNA polymerase, and the low number of PCR cycles all contribute to the high mutation efficiency and decreased potential for random mutations during the reaction.

\* Patent Pending.

‡ See *Related Stratagene Products*.

¶ *PfuTurbo* DNA polymerase has 6-fold higher fidelity in DNA synthesis than *Taq* DNA polymerase.

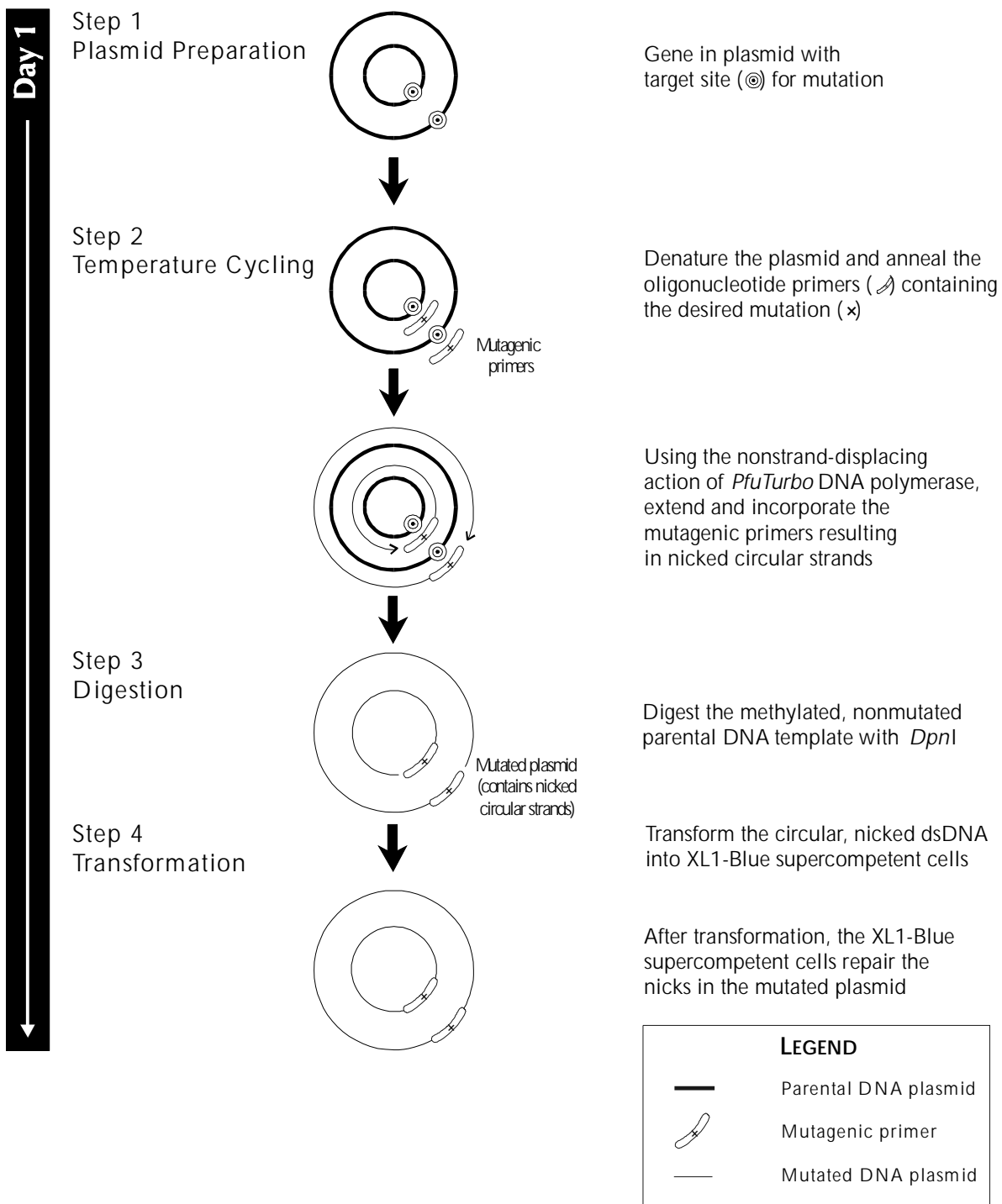


FIGURE 1 Overview of the QuikChange™ site-directed mutagenesis method.

To demonstrate the effectiveness of the QuikChange site-directed mutagenesis kit, the pWhitescript™ 4.5-kb control plasmid is used to test the efficiency of mutant plasmid generation. The pWhitescript 4.5-kb control plasmid contains a stop codon (TAA) at the position where a glutamine codon (CAA) would normally appear in the  $\beta$ -galactosidase gene of the pBluescript® II SK(-) phagemid (corresponding to amino acid 9 of the protein). Epicurian Coli XL1-Blue supercompetent cells transformed with this control plasmid appear white on LB-ampicillin agar plates (see *Preparation of Media and Reagents*), containing IPTG and X-gal, because  $\beta$ -galactosidase activity has been obliterated. The oligonucleotide control primers create a point mutation that reverts the T residue of the stop codon (TAA) in the  $\beta$ -galactosidase gene encoded on the pWhitescript 4.5-kb control template to the C residue to produce a glutamine codon (Gln, CAA). Following transformation, colonies can be screened for the  $\beta$ -galactosidase ( $\beta$ -gal<sup>+</sup>) (blue) phenotype.

## MATERIALS PROVIDED

Material provided <sup>a</sup>	Quantity
<i>PfuTurbo</i> ™ DNA polymerase (2.5 U/ $\mu$ l)	30 reactions
10 $\times$ reaction buffer <sup>b</sup>	1 ml
<i>Dpn</i> I restriction enzyme (10 U/ $\mu$ l)	300 U
Oligonucleotide control primer #1 [34-mer (100 ng/ $\mu$ l)] 5' CCA TGA TTA CGC CAA GCG CGC AAT TAA CCC TCA C 3'	750 ng
Oligonucleotide control primer #2 [34-mer (100 ng/ $\mu$ l)] 5' GTG AGG GTT AAT TGC GCG CTT GGC GTA ATC ATG G 3'	750 ng
pWhitescript™ 4.5-kb control plasmid (5 ng/ $\mu$ l)	50 ng
dNTP mix <sup>c</sup>	30 $\mu$ l
Epicurian Coli® XL1-Blue supercompetent cells <sup>d</sup>	8 $\times$ 200 $\mu$ l
pUC18 control plasmid (0.1 ng/ $\mu$ l in TE buffer <sup>b</sup> )	10 $\mu$ l

<sup>a</sup> The QuikChange site-directed mutagenesis kit contains enough reagents for 25 test reactions and 5 control reactions.

<sup>b</sup> See *Preparation of Media and Reagents*.

<sup>c</sup> Thaw the dNTP mix once, prepare single-use aliquots, and store the aliquots at -20°C. **Do not subject the dNTP mix to multiple freeze-thaw cycles.**

<sup>d</sup> Genotype: *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lac<sup>h</sup>Z $\Delta$ M15 Tn10* (Tet<sup>r</sup>)]<sup>c</sup>

## ADDITIONAL MATERIALS REQUIRED

Falcon® 2059 polypropylene tubes (15 ml)

5-Bromo-4-chloro-3-indoyl- $\beta$ -D-galactopyranoside (X-gal)<sup>‡</sup>

Isopropyl-1-thio- $\beta$ -D-galactopyranoside (IPTG)<sup>‡</sup>

<sup>‡</sup> See *Related Stratagene Products*.

## PRIMER DESIGN

**Note** *Stratagene offers Custom Oligonucleotide Synthesis,† a service which synthesizes custom oligodeoxynucleotides to meet specific research needs. Polyacrylamide gel electrophoresis (PAGE) purification† and 5′ phosphorylation† are also available. Please contact our Technical Services Department at the toll-free numbers listed on the inside front cover of this instruction manual or your local distributor for additional information or fax a typed 5′ to 3′ sequence to (619) 535-0045 (Attention: Custom Oligo Department).*

Mutagenic primers introduce specific experimental mutations. The mutagenic oligonucleotide primers for use in this protocol must be designed individually according to the desired mutation. The following considerations should be made for designing mutagenic site and selection primers:

1. Both the mutagenic primers must contain the desired mutation and anneal to the same sequence on opposite strands of the plasmid.
2. Primers should be between 25 and 45 bases in length, and the melting temperature ( $T_m$ ) of the primers should be greater than or equal to 78°C. The following formula is commonly used for estimating the  $T_m$  of primers:

$$T_m = 81.5 + 0.41(\%GC) - 675 / N - \% \text{ mismatch}$$

where  $N$  is the primer length in base pairs.

3. The desired mutation (deletion or insertion) should be in the middle of the primer with ~10–15 bases of correct sequence on both sides.
4. The primers optimally should have a minimum GC content of 40% and should terminate in one or more C or G bases.
5. Primers need not be 5′ phosphorylated but **must** be purified either by fast polynucleotide liquid chromatography (FPLC) or by polyacrylamide gel electrophoresis (PAGE). Failure to purify the primers results in a significant decrease in mutation efficiency.
6. It is important to keep primer concentration in excess. Stratagene suggests varying the amount of template while keeping the concentration of the primer constantly in excess.

† See *Related Stratagene Products*.

## TRANSFORMATION GUIDELINES

It is important to store the Epicurian Coli XL1-Blue supercompetent cells at  $-80^{\circ}\text{C}$  to prevent a loss of efficiency. For best results, please follow the directions outlined in the following sections.<sup>7</sup>

### Storage Conditions

The Epicurian Coli XL1-Blue supercompetent cells are very sensitive to even small variations in temperature and must be stored at the bottom of a  $-80^{\circ}\text{C}$  freezer. Transferring tubes from one freezer to another may result in a loss of efficiency. The Epicurian Coli XL1-Blue supercompetent cells should be placed at  $-80^{\circ}\text{C}$  directly from the dry ice shipping container. Cells stored in this manner should retain their guaranteed efficiency for 6 months.

### Aliquoting Cells

When aliquoting, keep the Epicurian Coli XL1-Blue supercompetent cells on ice at all times. It is essential that the Falcon® 2059 polypropylene tubes are placed on ice before the cells are thawed and that the cells are aliquoted directly into the prechilled tubes.

### Use of Falcon 2059 Polypropylene Tubes

It is important that Falcon 2059 polypropylene tubes are used for the transformation protocol because the incubation period during the heat-pulse step is critical and has been calculated for the thickness and shape of the Falcon 2059 polypropylene tubes.

### Length of the Heat Pulse

There is a defined "window" of highest efficiency for the Epicurian Coli XL1-Blue supercompetent cells resulting from the heat pulse in step 3 of the transformation protocol. Optimal efficiencies are observed when cells are heat pulsed for 45 seconds. Heat pulsing for at least 45 seconds is recommended to allow for slight variations in the length of incubation. Efficiencies decrease sharply when pulsing for  $<30$  seconds or for  $>45$  seconds.

### Preparing the Agar Plates for Color Screening

Prepare the LB-ampicillin agar plates for blue–white color screening 30 minutes prior to plating the transformations. Pipet 20  $\mu\text{l}$  of 10% (w/v) X-gal and 20  $\mu\text{l}$  of 100 mM IPTG into a 100- $\mu\text{l}$  pool of NZY<sup>+</sup> broth (see *Preparation of Media and Reagents*), and then spread the mixture across the plate. Prepare the X-gal in dimethylformamide (DMF). Prepare the IPTG in sterile dH<sub>2</sub>O. Do not mix the IPTG and the X-gal before pipetting them into the pool of NZY<sup>+</sup> broth because these chemicals may precipitate.

## PROTOCOL

### Setting Up the Reactions

**Note** *To maximize temperature-cycling performance, Stratagene strongly recommends using Thin-Wall Tubes (see Related Stratagene Products), which are optimized and tested to ensure ideal contact with the temperature cycler's temperature blocks.*

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 *Primer Design*).
2. Prepare the control reaction as indicated below:

5  $\mu$ l of 10 $\times$  reaction buffer  
2  $\mu$ l (10 ng) of pWhitescript™ 4.5-kb control plasmid (5 ng/ $\mu$ l)  
1.25  $\mu$ l (125 ng) of oligonucleotide control primer #1 [34-mer (100 ng/ $\mu$ l)]  
1.25  $\mu$ l (125 ng) of oligonucleotide control primer #2 [34-mer (100 ng/ $\mu$ l)]  
1  $\mu$ l of dNTP mix  
Double-distilled water (ddH<sub>2</sub>O) to a final volume of 50  $\mu$ l

Then add

1  $\mu$ l of *PfuTurbo* DNA polymerase (2.5 U/ $\mu$ l)

3. Prepare the sample reaction(s) as indicated below:

**Note** *Stratagene recommends setting up a series of sample reactions using various concentrations of dsDNA template ranging from 5 to 50 ng (e.g., 5, 10, 20, and 50 ng of dsDNA template) while keeping the primer concentration constant.*

5  $\mu$ l of 10 $\times$  reaction buffer  
X  $\mu$ l (5–50 ng) of dsDNA template  
X  $\mu$ l (125 ng) of oligonucleotide primer #1  
X  $\mu$ l (125 ng) of oligonucleotide primer #2  
1  $\mu$ l of dNTP mix  
ddH<sub>2</sub>O to a final volume of 50  $\mu$ l

Then add

1  $\mu$ l of *PfuTurbo* DNA polymerase (2.5 U/ $\mu$ l)

4. Overlay each reaction with 30  $\mu$ l of mineral oil.

TABLE I

## Cycling Parameters for the QuikChange Site-Directed Mutagenesis Method

Segment	Cycles	Temperature	Time
1	1	95°C	30 seconds
2	12–18	95°C	30 seconds
		55°C	1 minute
		68°C	2 minutes/kb of plasmid length

## Cycling the Reactions

1. Cycle each reaction using the cycling parameters outlined in Table I. (For the control reaction, use a 12-minute extension time and run the reaction for 12 cycles.)
2. Adjust segment 2 of the cycling parameters in accordance with the type of mutation desired (see the following table):

Type of mutation desired	Number of cycles
Point mutations	12
Single amino acid changes	16
Multiple amino acid deletions or insertions	18

3. Following temperature cycling, place the reaction on ice for 2 minutes to cool the reaction to  $\leq 37^{\circ}\text{C}$ .

**Note** To check for sufficient amplification, electrophorese 10  $\mu\text{l}$  of the amplified product on a 1% agarose gel.

## Digesting the Products

**Note** It is important to insert the pipet tip below the mineral oil overlay when adding the Dpn I restriction enzyme to the reaction tubes during the digestion step or when transferring the 1  $\mu\text{l}$  of the Dpn I-treated DNA required for the transformation reaction. Stratagene suggests using specialized aerosol-resistant pipet tips (see Related Stratagene Products), which are small and pointed, to facilitate this process.

1. Add 1  $\mu\text{l}$  of the Dpn I restriction enzyme (10 U/ $\mu\text{l}$ ) directly to each amplification reaction below the mineral oil overlay using a small, pointed pipet tip.
2. Gently and thoroughly mix each reaction mixture by pipetting the solution up and down several times. Spin down the reaction mixtures in a microcentrifuge for 1 minute and immediately incubate each reaction at 37°C for 1 hour to digest the parental (i.e., the nonmutated) supercoiled dsDNA.

## Transforming into Epicurian Coli XL1-Blue Supercompetent Cells

**Note** *Please read the Transformation Guidelines before proceeding with the transformation protocol.*

1. Gently thaw the Epicurian Coli XL1-Blue supercompetent cells on ice. For each control and sample reaction to be transformed, aliquot 50  $\mu\text{l}$  of the supercompetent cells to a *prechilled* Falcon 2059 polypropylene tube.
2. Transfer 1  $\mu\text{l}$  of the *Dpn* I-treated DNA from each control and sample reaction to separate aliquots of the supercompetent cells.

**Note** *Carefully remove any residual mineral oil from the pipet tip before transferring the Dpn I-treated DNA to each reaction.*

Swirl the transformation reactions gently to mix and incubate the reactions on ice for 30 minutes.

As an optional step, verify the transformation efficiency of the Epicurian Coli XL1-Blue supercompetent cells by adding 1  $\mu\text{l}$  of the pUC18 control plasmid (0.1 ng/ $\mu\text{l}$ ) to a 50- $\mu\text{l}$  aliquot of the supercompetent cells and incubating as indicated above.

3. Heat pulse the transformation reactions for 45 seconds at 42°C and then place the reactions on ice for 2 minutes.

**Note** *This heat pulse has been optimized for the Falcon 2059 polypropylene tubes.*

4. Add 0.5 ml of NZY<sup>+</sup> broth preheated to 42°C and incubate the transformation reactions at 37°C for 1 hour with shaking at 225–250 rpm.

5. Immediately plate the transformation reactions as follows:

- a. Plate 250  $\mu\text{l}$  of the control transformation reaction and only 5  $\mu\text{l}$  of the pUC18 control transformation reaction (if performed) on LB–ampicillin agar plates that have been prepared with 20  $\mu\text{l}$  of 10% (w/v) X-gal and 20  $\mu\text{l}$  of 100 mM IPTG (see *Preparing the Agar Plates for Color Screening*).

**Note** *When plating the 5  $\mu\text{l}$  of the pUC18 control transformation reaction, increase the volume of the transformation mixture to be plated to a total volume of 200  $\mu\text{l}$  using NZY<sup>+</sup> broth.*

- b. Plate the entire volume of each sample transformation reaction on agar plates containing the appropriate antibiotic for the plasmid vector.
6. Incubate the transformation plates at 37°C for >16 hours.

### ***Expected Results for the Control Transformations***

The expected colony number should be between 50 and 800 colonies. Greater than 80% of the mutagenized control colonies should contain the mutation and appear as blue colonies on agar plates containing IPTG and X-gal.

**Note** *The mutagenesis efficiency (ME) for the pWhitescript 4.5-kb control plasmid is calculated by the following formula:*

$$ME = \frac{\text{Number of blue colony forming units (cfu)}}{\text{Total number of colony forming units (cfu)}} \times 100\%$$

If transformation of the pUC18 control plasmid was performed, the transformation efficiency should be >250 colonies (>10<sup>8</sup> cfu) with >98% having the blue phenotype.

**Note** *The insert of interest should be sequenced prior to further experimentation to ensure the most accurate results.*

## TROUBLESHOOTING

When used according to the guidelines outlined in this instruction manual, Stratagene's kit will provide a reliable means to conduct site-directed mutagenesis using dsDNA templates. Undoubtedly, there will be variations in the base composition and length of the DNA template and in the thermal cycler (see *Related Stratagene Products*) that may contribute to differences in mutagenesis efficiency. Stratagene provides the following guidelines for troubleshooting these variations.

Observation	Possible cause(s)	Suggestion(s)
Low transformation efficiency or low colony number	Too much mineral oil pipetted with the <i>Dpn</i> I-treated DNA while transferring to the transformation reaction	Using the smallest pipet tips available, insert the pipet tip completely below the mineral layer overlay and clear the pipet tip while submerged beneath the mineral oil overlay before collecting the sample
	Insufficient amount of mutant DNA synthesized in the reaction	Increase the amount of the <i>Dpn</i> I-treated DNA used in the transformation reaction to 4 $\mu$ l
	Insufficient amount of DNA template used in the reaction	Visualize the DNA template on a gel to verify the quantity and quality
Low mutagenesis efficiency or low colony number with the control reaction	Differences in thermal cyclers may contribute to variations in ramping efficiencies	Adjust the cycling parameters for the control reaction and repeat the protocol for the sample reactions
	Little or no linear amplification products	Following temperature cycling, resolve a sample of the control reaction by electrophoresis on an agarose gel; if no product is observed at 4.5 kb, adjust the cycling parameters for the control reaction
	Supercompetent cells stored at an improper temperature	Store the supercompetent cells immediately at the bottom of a $-80^{\circ}\text{C}$ freezer (see also <i>Transformation Guidelines</i> )
	Insufficient amounts of X-gal and IPTG on the agar plates	Prepare the LB-ampicillin agar plates for the transformed control cells by pipetting 20 $\mu$ l of 10% (w/v) X-gal (prepared in DMF) and 20 $\mu$ l of 100 mM IPTG (prepared in filter-sterilized $\text{dH}_2\text{O}$ ) into a 100- $\mu$ l pool of NZY <sup>+</sup> broth and then spreading the mixture across the plate (see <i>Preparing the Agar Plates for Color Screening</i> )

(table continues on the next page)

(table continues from the previous page)

Observation	Possible cause(s)	Suggestion(s)
Low mutagenesis efficiency with the sample reaction(s)	Subjecting the dNTP mix to multiple freeze-thaw cycles	<p>For best visualization of the blue (<math>\beta</math>-gal<sup>+</sup>) phenotype, the control plates must be incubated for at least 16 hours at 37°C</p> <p>Thaw the dNTP mix once, prepare single-use aliquots, and store the aliquots at -20°C. Do not subject the dNTP mix to multiple freeze-thaw cycles</p>
	Differences in thermal cyclers may contribute to variations in ramping efficiencies	Adjust the cycling parameters for the sample reaction
	Improper mixing of reagents	Add the <i>Dpn</i> I restriction enzyme below the mineral oil overlay in the digestion step and ensure proper mixing of all components in the reaction especially the <i>Dpn</i> I
	The amplification reaction contains too much DNA template	The <i>Dpn</i> I must be able to completely digest the parental template in the time allotted for the digestion; repeat the digestion if necessary
False positives	Subjecting the dNTP mix to multiple freeze-thaw cycles	Thaw the dNTP mix once, prepare single-use aliquots, and store the aliquots at -20°C. Do not subject the dNTP mix to multiple freeze-thaw cycles
	Quality of the primers is poor	Radiolabel the primers and check for degradation on an acrylamide gel or resynthesize the primers
	False priming	Increase the stringency of the reaction by increasing the annealing temperature to within 5°C of the melting temperature of the mutation primers

## PREPARATION OF MEDIA AND REAGENTS

### LB Agar (per Liter)

10 g of NaCl  
10 g of tryptone  
5 g of yeast extract  
20 g of agar  
Add deionized H<sub>2</sub>O to a final volume of  
1 liter  
Adjust pH to 7.0 with 5 N NaOH  
Autoclave  
Pour into petri dishes (~25 ml/100-mm  
plate)

### LB-Ampicillin Agar (per Liter)

(Use for reduced satellite colony  
formation)  
1 liter of LB agar  
Autoclave  
Cool to 55°C  
Add 50 mg of filter-sterilized ampicillin  
Pour into petri dishes (~25 ml/100-mm  
plate)

### NZY+ Broth (per Liter)

10 g of NZ amine (casein hydrolysate)  
5 g of yeast extract  
5 g of NaCl  
Adjust to pH 7.5 using NaOH  
Autoclave  
Add the following supplement prior to use  
12.5 ml of 1 M MgCl<sub>2</sub> and 12.5 ml of  
1 M MgSO<sub>4</sub>  
10 ml of a 2 M filter-sterilized  
glucose solution or 20 ml of  
20% (w/v) glucose  
Filter sterilize

### 10× Reaction Buffer

100 mM KCl  
100 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
200 mM Tris-HCl (pH 8.8)  
20 mM MgSO<sub>4</sub>  
1% Triton® X-100  
1 mg/ml nuclease-free bovine serum  
albumin (BSA)<sup>‡</sup>

### TE Buffer

10 mM Tris-HCl<sup>‡</sup> (pH 7.5)  
1 mM EDTA<sup>‡</sup>

## RELATED STRATAGENE PRODUCTS

### Nucleic Acid Purification

StrataPrep™ plasmid miniprep kit [Catalog #400761 (50 preps) and #400763 (250 preps)]  
ClearCut™ miniprep kit [Catalog #400732 (50 minipreps) and #400733 (200 minipreps)]

### Fine Chemicals

X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) [Catalog #300200 (250 mg),  
#300201 (1 g), and #300204 (10 g)]  
IPTG (isopropyl-1-thio-β-D-galactopyranoside) [Catalog #300127 (1 g)]  
Tris-HCl [tris(hydroxymethyl)aminomethane hydrochloride] [Catalog #300181 (500 g) and  
#300182 (1 kg)]  
EDTA (ethylenediaminetetraacetic acid-disodium salt) [Catalog #300071 (250 g), #300072  
(500 g), and #300073 (1 kg)]  
Bovine Serum Albumin (nuclease free) (15 mg/ml) [Catalog #300041 (1 ml) and #300042  
(10 ml)]

<sup>‡</sup> See *Related Stratagene Products*.

## Equipment and Labware

- RoboCycler® Gradient 96 temperature cycler<sup>†</sup> [Catalog #400880 (120/100 V) and #400882 (230 V)]
- RoboCycler® 96 temperature cycler<sup>†</sup> [Catalog #400870 (120/100 V) and #400872 (230 V)]
- RoboCycler® Gradient 40 temperature cycler<sup>†</sup> [Catalog #400860 (120/100 V) and #400862 (230 V)]
- RoboCycler® 40 temperature cycler<sup>†</sup> [Catalog #400830 (120/100 V) and #400832 (230 V)]
- Aerosol-Resistant Pipet Tips {Catalog #410136 [10 µl (960 tips/package)] and #410137 [10 µl (1000 tips/package)]}
- Thin-Wall Tubes [Catalog #410090 (1000 600-µl nonsterile tubes) and #410091 (1000 200-µl nonsterile tubes)]
- Thin-Wall Tube Strips [Catalog #410092 (120 8-tube strips of 200-µl tubes) and #410096 (120 8-cap strips)]

## Synthetic Oligonucleotides

- Custom Oligonucleotide Synthesis (Catalog #912000)
- 5' Phosphorylation (Catalog #900675)
- PAGE Purification (Catalog #900674)

## Competent Cells

- Epicurian Coli® XL1-Blue supercompetent cells (Catalog #200236)

<sup>†</sup> See *Endnotes*.

## STRATAGENE DISTRIBUTORS

Country	Distributor name	Telephone number	Fax number
Argentina	Tecnolab S.A.	541 555 0010	541 553 3331
Australia	Integrated Sciences Toll free	(02) 9417 7866 (800) 252204	(02) 9417 5066
Austria	See inside front cover		
Brazil	Instrucom	11 5561 1771	11 530 0895
Canada	See inside front cover		
Denmark	AH Diagnostics	86 10 10 55	86 16 15 33
Egypt	Clinilab	202 3518763	202 3781507
Finland	Kebo Finland	358 9 804 551	358 9 804 55200
France	Ozyme	1 34 60 24 24	1 34 60 92 12
Germany	See inside front cover		
Hong Kong	Line Analytics Ltd.	2578 5839	2807 2674
India	Wipro Ltd.	91 11 3325677	91 11 3738675
Ireland	B.M. Brownes Ltd.	353 1 295 3401	353 1 295 3818
Israel	Getter Photo Supplies (2000) Ltd.	3 576 1555	3 752 3620
Italy	Eppendorf s.r.l.	02-58.01.34.09	02-58.01.34.38
Japan	Funakoshi Co., Ltd. Toyobo Co., Ltd. (Tokyo) Toyobo Co., Ltd. (Osaka)—Main office	3 5684 1622 3 3660 4819 6 348 3785/6 348 3788	3 5684 1633 3 3660 4887 6 348 3322
Korea	Koram Biotech Corp.	2 556 0311	2 556 0828
Mexico	Bioselec	355 8928	556 6943
New Zealand	LabSupply Pierce (NZ) Ltd.	9 443 5867	9 444 7314
Norway	MedProbe A.S.	47 22 20 01 37	47 22 20 01 89
Portugal	Biocontec	1 361 3620	1 362 5615
Republic of China	Merck Taiwan Ltd.	886 2 2521 9331	886 2 2536 7734
Singapore	ITS Science & Medical Pte. Ltd.	273 0898	273 0810
South Africa	Whitehead Scientific Supplies	21 981 1560	21 981 5789
Spain	Cultek	91 729 03 33	91 358 17 61
Sweden	AH Diagnostics AB Toll free	86 80 08 45 800 10299	86 80 04 35
Switzerland	See inside front cover		
Thailand	ITS (Thailand) Co., Ltd.	2 308 0611	2 308 0612
United Kingdom	See inside front cover		
United States	See inside front cover		

## REFERENCES

1. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82: 488.
2. Vandeyar, M., Weiner, M. P., Hutton, C., and Batt, C. (1988) *Gene* 65: 129–133.
3. Sugimoto, M., Esaki, N., Tanaka, H., and Soda, K. (1989) *Anal. Biochem.* 179: 309–311.
4. Taylor, J. W., and Eckstein, F. (1985) *Nucleic Acids Res.* 13: 8764.
5. Papworth, C., Braman, J., and Wright, D. A. (1996) *Strategies* 9(1): 3–4.
6. Bergseid, M., Scott, B., Mathur, S., Nielson, K., Shoemaker, D., and Mathur, E. (1991) *Strategies* 4(3): 34–35.
7. Nelson, M., and McClelland, M. (1992) *Methods Enzymol.* 216: 279–303.

## ENDNOTES

† Practice of the patented Polymerase Chain Reaction (PCR) process requires a license. Stratagene's thermal cycler is an Authorized Thermal Cycler. Its use with Authorized Reagents provides a limited PCR license in accordance with the label rights accompanying such reagents. It may also be used with PCR licenses available from The Perkin-Elmer Corporation.

Epicurian Coli<sup>®</sup>, pBluescript<sup>®</sup>, and RoboCycler<sup>®</sup> are registered trademarks of Stratagene in the United States.

ClearCut, *PfuTurbo*, pWhitescript, QuikChange, and StrataPrep are trademarks of Stratagene.

Falcon<sup>®</sup> is a registered trademark of Becton Dickinson and Company.

Triton<sup>®</sup> is a registered trademark of Rohm and Haas Co.



## QuikChange™ Site-Directed Mutagenesis Kit

Catalog #200518

### QUICK-REFERENCE PROTOCOL

- Prepare the control and sample reaction(s) as indicated below:

**Note** *Stratagene recommends setting up a series of sample reactions using various concentrations ranging from 5 to 50 ng of dsDNA template (e.g., 5, 10, 20, and 50 ng of dsDNA template).*

#### Control Reaction

5  $\mu$ l of 10 $\times$  reaction buffer  
 2  $\mu$ l (10 ng) of pWhitescript™ 4.5-kb control template (5 ng/ $\mu$ l)  
 1.25  $\mu$ l (125 ng) of oligonucleotide control primer #1 [34-mer (100 ng/ $\mu$ l)]  
 1.25  $\mu$ l (125 ng) of oligonucleotide control primer #2 [34-mer (100 ng/ $\mu$ l)]  
 1  $\mu$ l of dNTP mix  
 ddH<sub>2</sub>O to a final volume of 50  $\mu$ l

#### Sample Reaction

5  $\mu$ l of 10 $\times$  reaction buffer  
 X  $\mu$ l (5–50 ng) of dsDNA template  
 X  $\mu$ l (125 ng) of oligonucleotide primer #1  
 X  $\mu$ l (125 ng) of oligonucleotide primer #2  
 1  $\mu$ l of dNTP mix  
 ddH<sub>2</sub>O to a final volume of 50  $\mu$ l

- Then add 1  $\mu$ l of *PfuTurbo* DNA polymerase (2.5 U/ $\mu$ l) to each control and sample reaction
- Overlay each reaction with 30  $\mu$ l of mineral oil
- Cycle each reaction using the cycling parameters outlined in the following table:

Segment	Cycles	Temperature	Time
1	1	95°C	30 seconds
2	12–18	95°C	30 seconds
		55°C	1 minute
		68°C	2 minutes/kb of plasmid length

- Adjust segment 2 of the cycling parameters in accordance with the type of mutation desired (see the table in step 2 of *Cycling the Reactions* in the instruction manual)
- Add 1  $\mu$ l of the *Dpn* I restriction enzyme (10 U/ $\mu$ l) below the mineral oil overlay
- Gently and thoroughly mix each reaction, spin down in a microcentrifuge for 1 minute, and immediately incubate at 37°C for 1 hour to digest the parental supercoiled dsDNA
- Transform 1  $\mu$ l of the *Dpn* I-treated DNA from each control and sample reaction into separate 50- $\mu$ l aliquots of Epicurian Coli® XL1-Blue supercompetent cells (see *Transforming into Epicurian Coli XL1-Blue Supercompetent Cells* in the instruction manual)