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Instruction Manual

GeneTailor™ Site-Directed Mutagenesis System

For standard and high-throughput in vitro site-directed mutagenesis

Catalog nos. 12397-014 and 12397-022

Version G
8 April 2004
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Table of Contents

Kit Contents and Storage

Shipping and Storage

The kit is shipped on dry ice. Store competent cells at -80° C. Store all other components at -20° C.

Kit Sizes and Components

The GeneTailor™ Site-Directed Mutagenesis System* is available in two sizes:

- Catalog no. 12397-014 — 16 reactions
- Catalog no. 12397-022 — 96 reactions

Note: Each kit provides enough reagents for the specified number of methylation reactions (16 or 96) on separate plasmids. Because only 2–5 µl of each 16-µl methylation reaction is used in a mutagenesis reaction, each methylation reaction provides enough methylated plasmid for extra mutagenesis reactions using the same plasmid.

The table below lists the components for each size:

Component	16-reaction kit	96-reaction kit
DNA Methylase (4 units/µl)	20 µl	110 µl
Methylation Buffer	30 µl	175 µl
200X SAM (3.2 M)	10 µl	25 µl
dNTP mix (10 mM each)	50 µl	200 µl
Control Plasmid (100 ng/µl)	10 µl	10 µl
Control Primers (10 µM each)	10 µl	10 µl
Sterile, Distilled Water	1500 µl	5000 µl
One-Shot® MAX Efficiency® DH5α™-T1^R	1 box (21 × 50 µl)	—
MAX Efficiency® DH5α™- T1^R	—	5 boxes (25 × 200 µl)

*Patent pending

One-Shot® MAX Efficiency® DH5α™-T1^R Components

The table below describes the items included in each box of One-Shot® MAX Efficiency® DH5α™-T1^R competent cells (Catalog no. 12297-016). The reagents provided are sufficient for 21 transformations.

Store at -80°C.

Component	Composition	Amount
DH5α™-T1^R Competent Cells	—	21 × 50 µl
SOC Medium	2% Tryptone 0.5% Yeast Extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	6 ml
pUC19	10 pg/µl	50 µl

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MAX Efficiency® DH5α™-T1^R Components

The table below describes the items included with each box of MAX Efficiency® DH5α™-T1^R competent cells (Catalog no. 12034-013). The reagents provided are sufficient for 100 transformations.

Store at -80°C.

Component	Composition	Amount
DH5α™-T1^R Competent Cells	—	5 × 200 µl
SOC Medium	2% Tryptone 0.5% Yeast Extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	6 ml
pUC19	10 pg/µl	100 µl

Introduction

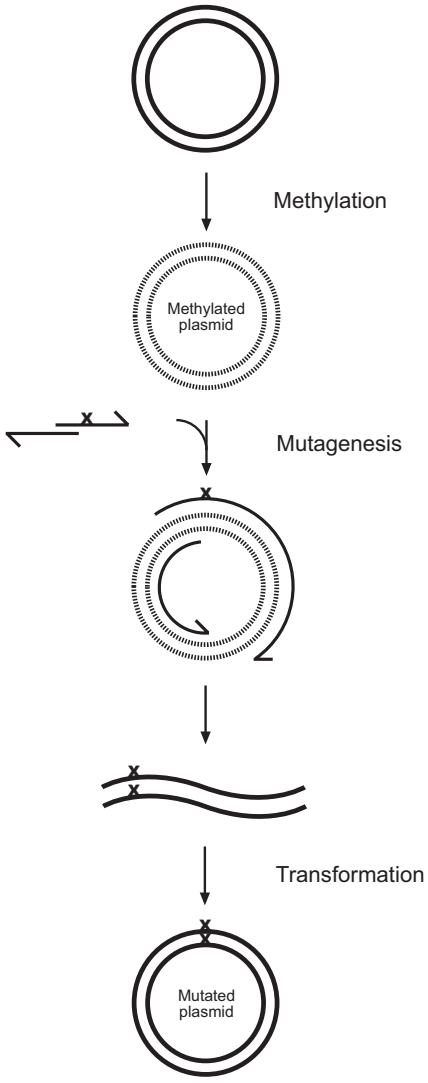
Overview

The GeneTailor™ Site-Directed Mutagenesis System is a simple and highly efficient method for standard and high-throughput *in vitro* site-directed mutagenesis. This unique system can generate base substitutions, deletions, or insertions of up to 21 nucleotides in DNA plasmids of up to 8 kb from any source, with no specialized vectors, host strains, or restriction sites required. Only one mutagenic oligonucleotide primer is required to generate a mutation site. No *in vitro* digestion step is required after the mutagenesis reaction, and no purification step is required after methylation or mutagenesis.

The high efficiency (>80%) and simplified protocols of this kit allow for the generation of site-directed mutants from the same or different target genes in a high-throughput format.

The GeneTailor™ Site-Directed Mutagenesis System relies on the inherent properties of two enzymes, DNA methylase and *Mcr*BC endonuclease, as shown in the workflow diagram below:

Workflow



1. Methylate plasmid DNA (isolated from any source) with DNA methylase at 37°C for 1 hour.
2. Amplify the plasmid in a mutagenesis reaction with two overlapping primers, one of which contains the target mutation. The product is linear, double-stranded DNA containing the mutation.
3. Transform the mutagenesis mixture into wild type *E. coli*. The host cell circularizes the linear mutated DNA, and *Mcr*BC endonuclease in the host cell digests the methylated template DNA, leaving only unmethylated, mutated product.

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Applications

In vitro site-directed mutagenesis can be used to:

- Study protein function
- Identify enzyme active sites
- Design new proteins

Target Plasmid and Primer Specifications

Introduction

This section describes the specifications for the DNA plasmids and overlapping primers to be used with the GeneTailor™ Site-Directed Mutagenesis System. You must design and order your primers separately.

Plasmid Specifications

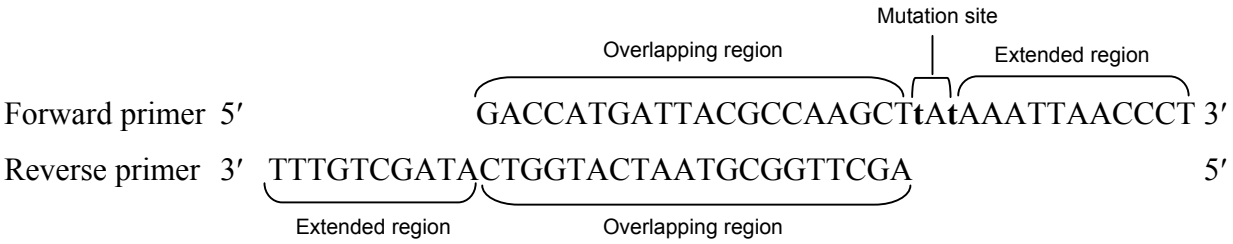
This kit has been tested using plasmids ranging in size from 2.4 kb to 8.0 kb. Plasmids may be isolated from any source. No special host strains, vectors, or restriction sites are required.

Primer Specifications

Follow the specifications below when designing your primers:

- Both primers (forward and reverse) should be approximately 30 nucleotides in length, not including the mutation site on the mutagenic primer
- Primers should have an overlapping region at the 5′ ends of 15–20 nucleotides, for efficient end-joining of mutagenesis product
- The mutation site should be located on only one of the primers, downstream from and adjacent to the overlapping region, and can be up to 21 bases (deletions, insertions, and/or any substitutions)
- On the mutagenic primer, there should be at least 10 nucleotides downstream of the mutation site for efficient annealing

The control primers included in the kit provide an example of possible primer designs. The control primers are shown below (note that the mutation can be located on either primer):



Ordering Custom Primers

Custom primers can be ordered directly from Invitrogen. Visit our Web site at www.invitrogen.com/oligos to order.

Control Plasmid and Primers

Control Plasmid

The control plasmid is a 3.4 kb plasmid containing the *lacZ*α gene, whose wild type produces blue colonies on plates containing LB/Amp/X-gal.

Control Primers

Two control primers are supplied with each kit:

- The forward (mutagenic) primer contains a two-base substitution that introduces a *Hind* III site and stop codon within the *lacZ*α gene. The stop codon generates a truncated LacZα protein and produces white colonies on plates containing X-gal.

  5′-GACCATGATTACGCCAAGCTTATAAATTAACCCT-3′ (34 bases)

- The reverse primer contains a 20-nucleotide sequence that is complementary to the forward primer, and includes 10 additional nucleotides at its 3′ end.

  5′-AGCTTGGCGTAATCATGGTCATAGCTGTTT-3′ (30 bases)

Methylation Reaction

Introduction

In this step, you methylate the plasmid for 1 hour. The DNA Methylase methylates cytosine residues within a specific sequence throughout the double-stranded DNA. The methylated DNA is subject to Mcr and Mrr restriction in *E. coli*, as described by Bandaru *et al* and Wyszynski *et al*.

Additional Materials Needed

- Target plasmid
- Water bath set at 37° C
- Sterile tubes

Note

Use no more than 100 ng of plasmid DNA per 16 µl of methylation reaction, as shown in the reaction volumes on the following page. Too much plasmid will reduce the efficiency of the methylation reaction.

Preparing 10X SAM

You should create a fresh dilution of 10X SAM from the kit-supplied 200X SAM and sterile, distilled water each time you perform the methylation procedure.

The following table provides example volumes for preparing 10X SAM:

	Number of Methylation Reactions		
Component	**6**	**12**	**18**
200X SAM	0.5 µl	1 µl	1.5 µl
Sterile, distilled water	9.5 µl	19 µl	28.5 µl
Total 10X SAM	10 µl	20 µl	30 µl

Important

10X SAM is not stable and will lose activity within a few hours after preparation. **Do not use** 10X SAM if it is more than a few hours old.

Continued on next page

Methylation Reaction, continued

Methylation Reaction

Use the amounts listed below as guidelines for preparing your methylation reactions—scale up or down as needed.

Note: The single reaction described below provides enough methylated plasmid for up to 8 mutagenesis reactions on the same target. The large-scale reaction provides enough methylated plasmid for up to 96 mutagenesis reactions on the same target.

Reagent	Single reaction	Large-scale reaction
Plasmid DNA	100 ng	1.4 µg
Methylation Buffer	1.6 µl	22.4 µl
10x SAM (see previous page)	1.6 µl	22.4 µl
DNA methylase (4 U/µl)	1.0 µl	14 µl
Sterile, distilled water	to 16 µl	to 220 µl

1. Combine reagents and incubate at 37°C for 1 hour.
2. After methylation, proceed to **Mutagenesis Reaction** on the next page.

Storage of Methylated Plasmids

The methylation reaction can be stored at –20° C. The methylated DNA will remain stable (no significant loss of mutagenesis efficiency) at this temperature for up to 3 months.

Mutagenesis Reaction

Introduction

In this step, you perform a mutagenesis reaction with the methylated plasmid, a mutagenic and a corresponding complementary primer, and one of the high fidelity, thermostable polymerases noted below.

Materials Needed

- Thermocycler
- Thermostable DNA polymerase (see below)
- Custom primers for your target plasmid (see page 5 for criteria)
- Methylation mix for target plasmid (from previous page)
- Control primers
- Methylation mix for control plasmid (from previous page)
- High-throughput reactions: 96-well plate

DNA Polymerase

For the mutagenesis reaction, use either Platinum® *Pfx* DNA Polymerase or Platinum® *Taq* DNA Polymerase High Fidelity, depending on the size of the target plasmid and the size of the reaction. These polymerases are available for purchase separately from Invitrogen (see the table below).

Reaction set-ups for these polymerases are provided on the following pages.

Note: The GeneTailor™ Site-Directed Mutagenesis System has been optimized for use with these polymerases; performance cannot be guaranteed with other DNA polymerases.

DNA Polymerase	Catalog numbers	Recommended plasmid size	Recommended reaction size
Platinum® *Pfx* DNA Polymerase	11708-013 (100 rxns) 11708-021 (250 rxns) 11708-039 (500 rxns)	<5 kb	Standard (50 µl)
Platinum® *Taq* DNA Polymerase High Fidelity	11304-011 (100 rxns) 11304-029 (500 rxns) 11304-102 (5000 rxns)	up to 8 kb	Standard (50 µl) or High Throughput (5 ml)

Amount of Plasmid

We recommend using 2 µl of methylation mixture (containing 12.5 ng of methylated plasmid) per 50 µl of mutagenesis reaction as a starting point. If you get very faint bands (see **Analyzing Mutagenesis Products** on page 11), up to 5 µl may be required.

Using more than 5 µl of methylation mix may decrease yield.

Continued on next page

Standard Reaction Setup— Platinum® *Taq* High Fidelity

The following 50-µl reaction mixture is recommended as a starting point when using Platinum® *Taq* DNA Polymerase High Fidelity with this kit. Prepare the mixture and perform cycling as described below.

Component	Volume	Final Concentration
10X High Fidelity PCR Buffer	5 µl	1X
10 mM dNTP	1.5 µl	0.3 mM each
50 mM $MgSO_4$*	1 µl	1 mM
Primers (10 µM each)	1.5 µl	0.3 µM each
Methylated DNA (12.5–31.25 ng)**	2–5 µl	As required
Platinum® *Taq* High Fidelity (5 U/µl)***	0.2–0.5 µl	1–2.5 units
Autoclaved, distilled water	to 50 µl	

*This is half the standard $MgSO_4$ concentration, because some Mg^{++} is carried over from the methlyation reaction.

In most cases, 2 µl of methylated DNA should be sufficient. For very faint bands (see **Analyzing Mutagenesis Products, following page), up to 5 µl may be required.

***For most targets, 1 unit is sufficient. In some cases, up to 2.5 units may be required.

High-Throughput Reaction Setup— Platinum® *Taq* High Fidelity

The following reaction mixture is large enough for 110 reactions of 50 µl each. Use only Platinum® *Taq* High Fidelity for high-throughput reactions.

Component	Volume	Final Concentration
10X High Fidelity PCR Buffer	550 µl	1X
10 mM dNTP	165 µl	0.3 mM each
50 mM $MgSO_4$	110 µl	1 mM
Platinum® *Taq* High Fidelity (5 U/µl)	21–55 µl	1–2.5 units/rxn
Autoclaved, distilled water	to 5.5 ml	

Prepare the mixture, and:

1. Aliquot 50 µl of reaction mixture into each well of a 96-well microplate.
2. Add 1.5 µl of 10 µM primer mix and 2–5 µl (12.5–31.25 ng) of methylated DNA to each well.
3. Thermocycle the plate as described below.

Cycling— Platinum® *Taq* High Fidelity

The cycling parameters below specify a 1-minute extension for each 1 kb of DNA. We recommend 20 cycles for optimal efficiency; additional cycles will produce better yield, but no increase in mutagenesis efficiency.

Temperature	Time	Cycles
94°C	2 minutes	1
94°C	30 seconds	
55°C	30 seconds	20
68°C	1 min/ 1 kb DNA	
68°C	10 minutes	1

Maintain the reaction at 4°C after cycling.

Continued on next page

Standard Reaction Setup— Platinum® *Pfx*

The following 50-µl reaction mixture is recommended as a starting point when using Platinum® *Pfx* DNA Polymerase with this kit. For high-throughput reactions, use Platinum® *Taq* High Fidelity (see previous page).

Components	Volume	Final Concentration
10X *Pfx* Amplification Buffer	5 µl	1X
10 mM dNTP	1.5 µl	0.3 mM each
50 mM $MgSO_4$	1 µl	1 mM
Primer mix (10 µM each)	1.5 µl	0.3 µM each
Methylated DNA (12.5–31.25 ng)*	2–5 µl*	As required
Platinum® *Pfx* DNA Polymerase (2.5 U/µl)**	0.4–1 µl	1.0–2.5 units
Autoclaved, distilled water	to 50 µl	

*In most cases, 2 µl of methylated DNA should be sufficient. For very faint bands (see **Analyzing Mutagenesis Products**, below), up to 5 µl may be required.

**For most targets 1 unit is sufficient. When amplifying longer targets (>3 kb), up to 2.5 units of enzyme may be required.

Cycling— Platinum® *Pfx*

The parameters below specify a 1-minute extension for each 1 kb of DNA. We recommend 20 cycles for optimal efficiency; additional cycles will produce better yield, but no increase in mutagenesis efficiency.

Temperature	Time	Cycles
94°C	2 minutes	1
94°C	30 seconds	
55°C	30 seconds	20
68°C	1 min/ 1 kb DNA	
68°C	10 minutes	1

Maintain the reaction at 4°C after cycling.

Analyzing Mutagenesis Products

After the reaction, analyze 10–20 µl of the product on a 1% agarose gel. Note that in some cases you may obtain high mutagenesis efficiency even with multiple or faint bands on an agarose gel. However, smeared bands may lead to low efficiency, and you should perform the mutagenesis reaction using another DNA polymerase.

Transformation into DH5α™-T1^R *E. Coli*

Note

One-Shot® MAX Efficiency® DH5α™-T1^R competent cells are supplied with the 16-reaction kit, and MAX Efficiency® DH5α™-T1^R competent cells are supplied with the 96-reaction kit. However, the following instructions and procedures can be performed using either type of cells.

General Handling

Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Transformation should be started immediately after thawing the cells on ice. **Mix by swirling or tapping the tube gently, not by pipetting or vortexing.**

Additional Materials Needed

- 37°C shaking and non-shaking incubator
- 10-cm diameter LB agar plates with appropriate antibiotic
- Ice bucket with ice
- 42°C water bath
- Single reactions: Test tube rack to hold all transformation tubes so that they can be put into the 42°C water bath at once.
- 96-well reactions: Multi-tip micropipettor
- 96-well reactions: 96-well growth block and air-porous tape

Before Starting

- Equilibrate a water bath to 42°C
- Warm a 200-µl vial of SOC medium to room temperature
- Spread X-Gal onto LB agar plates with antibiotic, if desired

Continued on next page

Transformation into DH5α™-T1^R *E. Coli*, Continued

Transformation Procedure—Single Reaction

1. Thaw on ice one 50-µl vial of DH5α™-T1^R cells for each transformation. Thaw for approximately 5–7 minutes (no more than 20 minutes). For multiple reactions (vials), number the tube caps.
2. Pipet 2 µl from each mutagenesis reaction mixture directly into each vial of cells and mix by tapping gently. **Do not mix by pipetting up and down.** Store the remaining mutagenesis reaction at -20°C.
3. Cap the vials, cover completely with ice, and incubate for 7–10 minutes.
4. Transfer vials to a test tube rack and incubate entire rack at once for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
5. Remove rack of vials from the 42°C bath and cover with ice for 1 minute.
6. Remove from ice, de-cap, and add **200 µl** of pre-warmed SOC medium to each vial. (SOC is a rich medium; sterile technique must be practiced to avoid contamination.)
7. Recap vials and place sideways in a microcentrifuge rack. Secure the vials with tape and shake at 37°C for exactly 1 hour at 225 rpm in a shaking incubator.
8. While the vials are shaking, label the LB agar plates and warm them for about 30 minutes in a 37°C incubator.
9. Aliquot 125 µl from each transformation vial onto a labeled plate. Do not allow cells to settle before aliquotting. If cells settle, mix by tapping the tube.
10. Gently spread the transformation reaction on the plate. Store the remaining transformation reaction at +4°C.
11. Invert the plates and incubate at 37°C for 16–20 hours.
12. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Continued on next page

Transformation into DH5α™-T1^R *E. Coli*, Continued

Transformation Procedure— 96-Well Plates

1. Pipet 2 µl of each mutagenesis reaction directly into each well of a 96-well growth block.
2. Cover with air-porous tape and chill the growth block containing the mutagenesis products at +4° C. Also chill a reservoir and micropipette tips at +4° C. Chill for 20 minutes.
3. While the growth block is cooling, thaw DH5α™-T1^R cells on ice for about 20 minutes, or until the cells are completely thawed.
4. Pour cells into the cold reservoir and aliquot 50 µl of cells into each well of the 96-well block using a multi-tip micropipettor with the chilled pipette tips.
5. Submerge the growth block in ice for 5minutes.
6. Remove the growth block from the ice and incubate in a 42°C water bath for exactly 30 seconds.
7. Remove the growth block from the water bath and place it in ice for 1minute.
8. Remove from ice and aliquot **200 µl** of pre-warmed SOC medium into each well. (SOC is a rich medium; sterile technique must be practiced to avoid contamination.)
9. Cover the growth block with air-porous tape and incubate in a 37° C shaker at 225 rpm for 1 hour.
10. While the growth block is shaking, label LB agar plates and warm them for about 30 minutes in a 37°C incubator.
11. Aliquot 125 µl from each well of the growth block onto a labeled LB agar plate. Do not allow the cells to settle before aliquotting.
12. Gently spread the transformation reaction on the plate. Store the remaining transformation reaction in the growth block at +4°C.
13. Invert the plates and incubate at 37°C for 16–20 hours.
14. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Methylation Control

Introduction

This control procedure can be used to test the methylation reaction.

Methylation Control Reaction

1. Set up two small-scale methylation reactions (see page 7)—one with 10X SAM and one without 10X SAM.
2. Dilute each reaction 25-fold with sterile, distilled water.
3. Transform each reaction into 50 µl of DH5α™-T1^R *E. Coli* (page 12).
4. Plate 125 µl of the cells with 10X SAM on an LB plate, and 25 µl of the cells without 10X SAM on a separate plate.
5. After incubation, determine the methylation efficiency using the following formula:

$$\% = \frac{\text{Total colonies x 5 (w/o SAM) - Total colonies (w/SAM)}}{\text{Total colonies x 5 (w/o SAM)}}$$

Expected efficiency: ≥ 90 percent.

Mutagenesis Control

Introduction

Use the control plasmid and primers to test the overall kit, including the methylation reaction and mutagenesis efficiency. The control plasmid is a 3.4 kb plasmid containing the *lacZ*α gene, whose wild type produces blue colonies on plates containing LB/Amp/X-gal.

The control mutagenic primer contains a two-base substitution that introduces a *Hind* III site and stop codon within the *lacZ*α gene. The stop codon generates a truncated LacZα protein and produces white colonies on plates containing X-gal.

Before Starting

For the mutagenesis control reaction, prepare your LB agar plates with 100 µg/ml Ampicillin and 400 µg/ml X-Gal (Catalog no. 15520-018) prior to transformation.

Mutagenesis Control Reaction

To test the efficiency of the overall kit using the control plasmid and primers:

1. Methylate the control plasmid as described on page 7.
2. Perform the mutagenesis reaction on page 9 using the methylated plasmid and the control primers.
3. Prepare the LB agar plates with X-Gal prior to transformation.
4. Transform the cells with the mutagenesis product (page 12).
5. After incubation, count blue and white colonies on the plates.

Results: The percentage of white colonies (mutants) should be ≥80%.

Note

Partially expressed *lacZα* genes containing the control mutation may result in colonies that are light blue on the inside and white on the outside. Light blue colonies should be considered mutant colonies and **not** wild type.

Competent Cell Control

Introduction

Use the pUC19 plasmid to test the efficiency of the competent cells included in the kit.

Competent Cell Control Reaction

If you do not obtain the expected number of colonies, we recommend that you test the efficiency of the competent cells. Transform DH5α™-T1^R with the supercoiled pUC19 plasmid supplied with the kit as described below.

1. Prepare LB agar plates containing 100 µg/ml Ampicillin.
2. Transform 5 µl (50 pg) of pUC19 into 50 µl of competent cells.
3. Incubate the vial on ice for 30 minutes.
4. Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
5. Remove vial from the 42°C bath and place on ice.
6. Add **250 µl** of pre-warmed SOC medium to each vial. (SOC is a rich medium; sterile technique must be practiced to avoid contamination.)
7. Place the vial in a microcentrifuge rack on its side and secure with tape to avoid loss of the vial. Shake the vial at 37°C for exactly 1 hour at 225 rpm in a shaking incubator.
8. Dilute the transformation reaction 1:100 and plate 30 µl on LB/Amp plates.
9. Incubate overnight at 37°C and count colonies. Calculate transformation efficiency using the formula below.

Calculation

Calculate the transformation efficiency as transformants per 1 µg of plasmid DNA. **Use the formula below to calculate transformation efficiency:**

$$\frac{\text{\# of colonies}}{\text{50 pg transformed DNA}} \times \frac{10^6\ \text{pg}}{\mu\text{g}} \times \frac{\text{300 µl total transformation volume}}{\text{30 µl plated}} \times 100 = \frac{\text{\# transformants}}{\mu\text{g plasmid DNA}}$$

Expected transformation efficiency: $\geq 1 \times 10^9$ cfu/µg supercoiled plasmid

Troubleshooting

Problem	Possible Cause	Potential Solution
High background when testing mutagenesis reaction product	Inactive DNA methylase or inactive SAM	Test the activity of the DNA methylase and 10X SAM using the methylation control reaction on page 15.
	Too much DNA	Use no more than 100 ng of DNA per 16 µl of methylation reaction.
	Denatured DNA	Purify new plasmid. We recommend using the S.N.A.P.™ MiniPrep Kit (K1900-01) for this purpose.
	Methylated plasmid is too old	Perform new methylation reaction. Methylated plasmid can be stored for up to 3 months at -20° C.
Too few colonies due to inefficient mutagenesis reaction	Too much methylated DNA used in reaction	Use no more than 5 µl of methylation product per 50 µl mutagenesis reaction.
	Not enough methylated DNA used in the reaction	Use 2–5 µl of methylation product per 50 µl mutagenesis reaction.
	DNA polymerase not optimal for plasmid	Platinum® *Pfx* is recommended for <5 kb plasmids. Platinum® *Taq* High Fidelity is recommended for plasmids up to 8 kb in size, and for high-throughput reactions (see page 10). Platinum® *Pfx* is a proofreading enzyme that offers higher fidelity than any other DNA polymerase; however, it is not as robust as Platinum® *Taq* High Fidelity. If you are working with a difficult-to-amplify template, Platinum® *Taq* High Fidelity is recommended.
	Annealing temperature is too low.	The annealing temperature should be no lower than 55° C.
	Extension time is too short.	We recommend an extension time of 1 min/1 kb. However, you should experiment with different extension times depending on your plasmids.
	Too much Mg^{++}	The methlyation mixture contains some Mg^{++}, which is carried over into the mutagenesis reaction. Try using half the recommended amount of Mg^{++} for your DNA polymerase in the mutagenesis reaction.
	3′ region of mutagenic primer is too short	The mutagenic primer should have at least 10 nucleotides downstream of the mutation site for optimal annealing.
	Overlapping 5′ region of primers is too long or too short	The 5′ overlapping region should contain 15–20 nucleotides. If this region is too long, primer self-annealing may occur. If it is too short, annealing efficiency between the overlapping regions may be compromised.
	Poor primer quality	Synthesize new primers or purify primers.

Product Qualification

Product Qualification

The GeneTailor™ Site-Directed Mutagenesis System is tested using the control plasmid and primers provided in the kit, as described on page 15. Mutagenesis efficiency should be ≥ 80%. The methlyation reaction is tested separately by methylating plasmids with and without 10X SAM, as described on page 15. The methylation efficiency should be ≥ 90%.

Competent *E. coli* Qualification

All competent cells are tested for transformation efficiency using the control plasmid. Transformed cultures are plated on LB plates containing 100 µg/ml Ampicillin and the transformation efficiency is calculated. Test transformations are performed in duplicate. Transformation efficiency should be ~1 x 10^9 cfu/µg DNA.

In addition, untransformed cells are tested for appropriate antibiotic sensitivity and lack of phage contamination. DH5α™-T1^R is also tested for resistance to phage T5, a standard test for resistance to phage T1.

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