6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Site-Directed Mutagenesis Kit

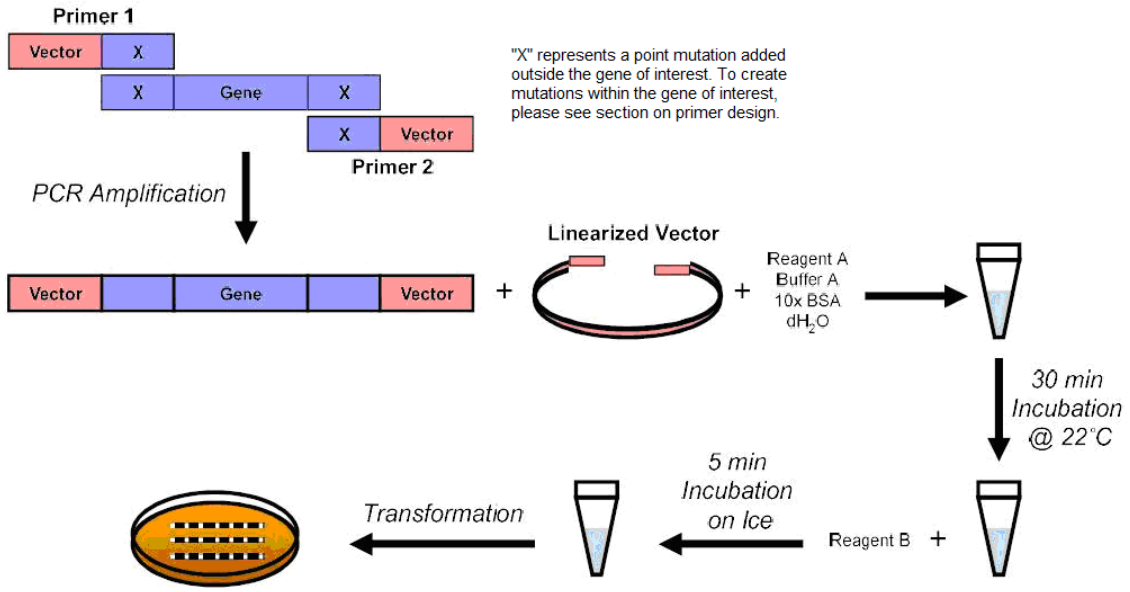
Catalog #: 20140 (20 reactions)

DESCRIPTION: The **Site-Directed Mutagenesis Kit** is a simple and highly efficient method to insert any mutation (point, insert, deletion) into a vector, without time-consuming ligation steps.

- ✓ No ligation steps required
- ✓ Multiple PCR inserts can be added in a single step
- ✓ Efficiencies of >90% are typical
- ✓ Go from PCR product to transforming cells in under an hour!
- ✓ Suitable for fixing or adding point mutations, insertions, or deletions
- ✓ Multiple PCR inserts or mutations can be added in a single step
- ✓ Available with or without competent *E. coli* cells for maximum flexibility

PROTOCOL OVERVIEW:

Mutations are easily generated in the gene of interest using appropriate primers and PCR amplification. This purified PCR product is incubated with restriction enzyme-digested vector and Reagent A of the Site-Directed Mutagenesis Kit. Multiple DNA fragments or mutations may be inserted in a single step. Following a brief incubation with Reagent B, the annealed vector is ready for transformation.



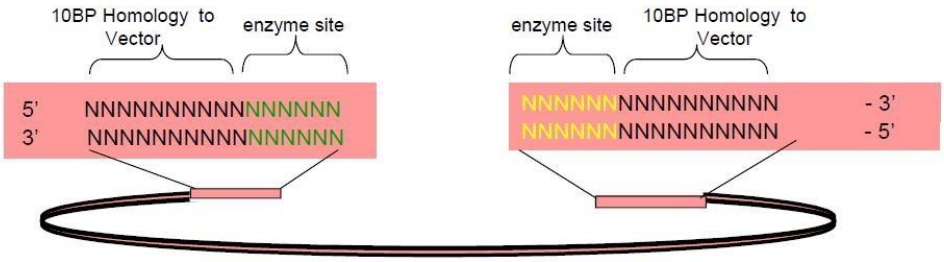
6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

COMPONENTS: Reagent A, Reagent B, Buffer A, 10X BSA (0.1% w/v), distilled water

	Cat. #	Amount
Including competent E. coli cells	20130	10 reactions
	20140	20 reactions
	20150	40 reactions
	20160	100 reactions
Not including competent E. coli cells	20170	10 reactions
	20180	20 reactions
	20190	40 reactions
	20195	100 reactions

STORAGE:
Store competent cells at -80°C; all other components may be stored at -20°C or -80°C. Avoid freeze/thaw cycles. Upon first thaw, store all reagents in single use aliquots.

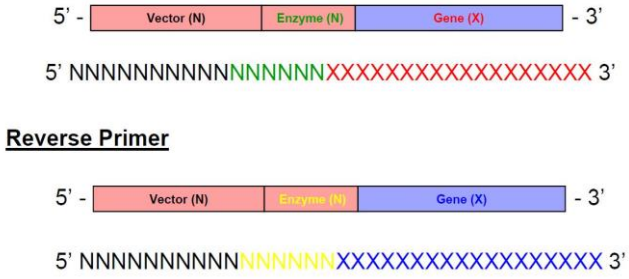
PRIMER DESIGN:
Proper primer design is critical for successful PCR and transformation steps. Primers for the gene of interest should contain the restriction site as well as at least 10 nucleotides of sequence homologous to the vector sequence. Using two different restriction enzymes will allow directional cloning of the insert. *Note : once purified, the digested vector can be used with multiple inserts if the primers for insert amplification are designed correctly.*



6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

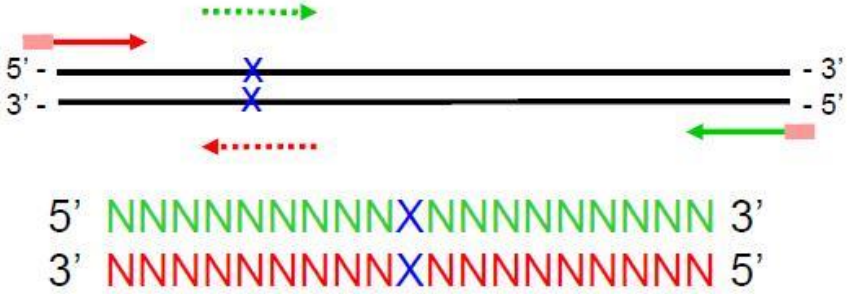
To make primers for PCR amplification of the gene or sequence of interest, at least 18 nucleotides of homology to the desired gene sequence is recommended.

Forward Primer

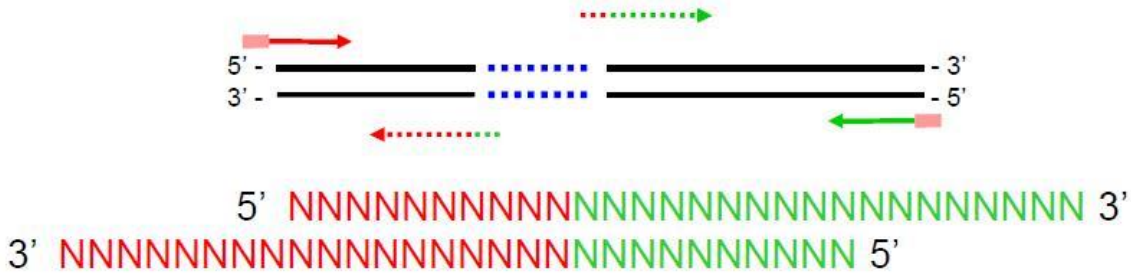


Reverse Primer

Point Mutation:
The interior primers should be designed with at least a 9-10 nucleotide overlap on each side of the mutated base(s).



Deletion Mutation:
The interior primer pair must be carefully designed with at least a 10 nucleotide overhang of the sequence on either side of the sequence to be deleted.



6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

PROTOCOL:

1) Linearize the vector using either one or two restriction enzymes and gel purify the fragment. *Note: Gel purification of the DNA fragment is critical for the reduction of false positives. We recommend using a commercial spin column extraction kit*.

2) Design each of the gene-specific primers; for optimal results, 15-18 base pair of overlap among DNA fragments is recommended. See section above for suggestions on primer design.

3) Amplify the gene by PCR, following manufacturer's instructions.

4) Isolate the PCR products by gel extraction (see note in step 1) and measure the concentration of the PCR product.

5) Prepare the 10 µL reaction mixture below using the PCR products at a 1:1 molar ratio to the linearized vector. Incubate at 22°C for 30 minutes

Reagent	**Amount**
linearized vector (100-200 ng)	x µl
PCR insert 1	y µl
PCR insert 2	z µl
10X BSA	1 µl
Reagent A	1 µl
Buffer A	1 µl
Distilled water	up to 10 µl
Total	**10 µl**

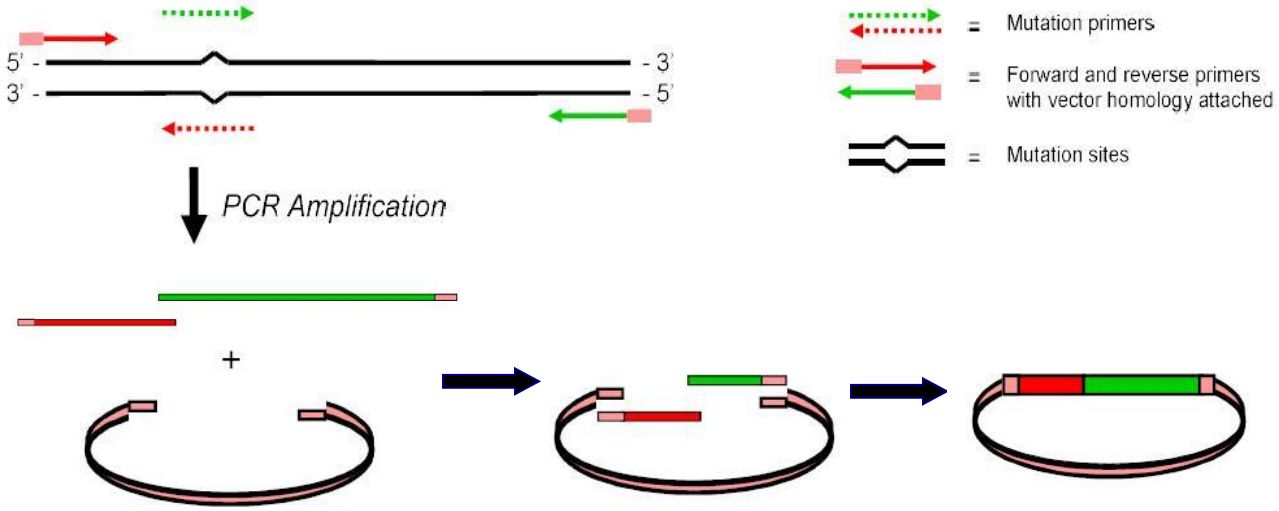
6) Add 1 µl Reagent B. Incubate microfuge tube on ice for 5 minutes.

7) Proceed with transformation using standard protocols, using the entire reaction mixture. **Note**: See Supplemental Instructions for Standard Transformation Protocol for details.

Inserting or "Fixing" mutations in DNA fragments

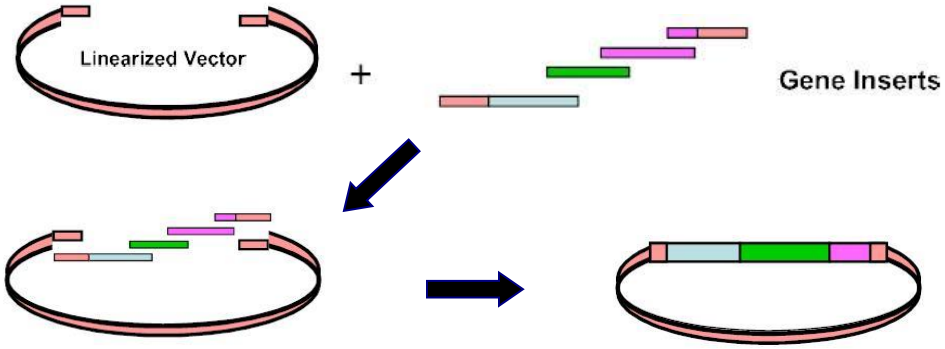
The Site-Directed Mutagenesis Kit can also be designed to add or "fix" mutations within a gene of interest, using the protocol for multiple DNA inserts (above). We recommend the two PCR products share at least 18 nucleotides of homology.

6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com



Insertion of multiple DNA fragments

The Site-Directed Mutagenesis Kit allows for the insertion of multiple DNA fragments into the desired vector using only a single reaction.



Note: Addition of more than 2 inserts in a single reaction may result in decreased cloning efficiency. PCR amplification can be used to combine fragments and minimize the number of inserts in a single reaction.

6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Supplemental Instructions for Standard Transformation Protocol

1) Place ligation reactions on ice to chill.
2) Thaw competent cells on ice.
3) Mix reaction with competent cells.
4) Incubate on ice for 30 minutes.
5) Heat shock at 42°C for 40 seconds.
6) Incubate on ice for 1 minute.
7) Add 1 mL LB (SOC media is better).
8) Shake for 1 hour at 37°C.
9) Spin down cells to pellet
10) Plate on LB agar selection plates.