BPS Bioscience

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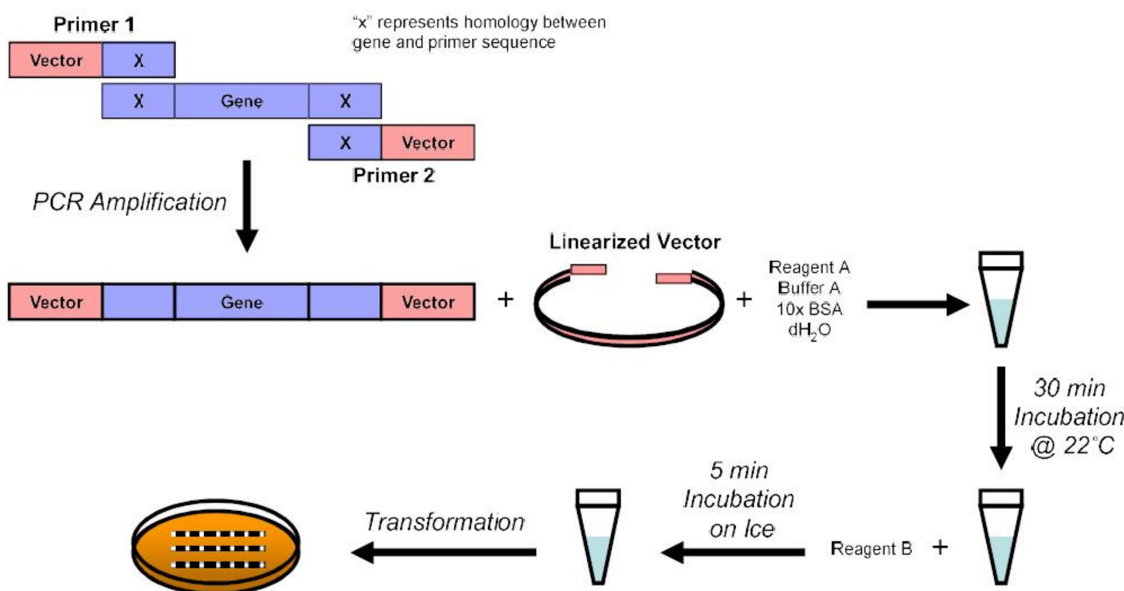
Data Sheet

Quick PCR™ Cloning Kit

DESCRIPTION: The **Quick PCR™ Cloning Kit** is a simple and highly efficient method to insert any gene or DNA fragment 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
- ✓ Available with or without competent *E. coli* cells for maximum flexibility

OVERVIEW:
A primer set homologous to the gene of interest and the vector is used to amplify a PCR product. This purified PCR product is incubated with restriction enzyme-digested vector and Reagent A of the Quick PCR™ cloning kit. Following a brief incubation with Reagent B, the annealed vector is ready for transformation.



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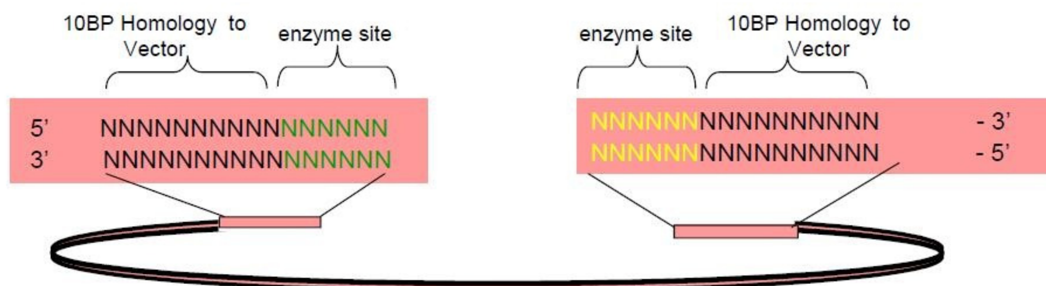
COMPONENTS: Reagent A, Reagent B, Buffer A, 10X BSA (0.1% w/v), distilled water.

	Cat. #	Amount
Including competent E. coli cells	20030	10 reactions
	20040	20 reactions
	20050	40 reactions
	20060	100 reactions
Not including competent E. coli cells	20070	10 reactions
	20080	20 reactions
	20090	40 reactions
	20100	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.*



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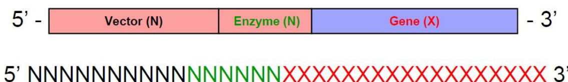
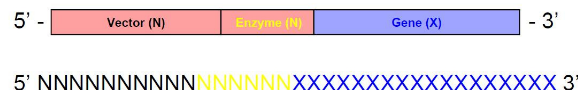
To make primers for PCR amplification of the gene or sequence of interest, at least 18 nucleotides of homology to the desired gene sequence is recommended.

Forward Primer



Reverse Primer



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 the gene-specific primers, including >15 base pair homology to the end of the linearized vector. See section above for suggestions on primer design.

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

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

5) Set up a 10 µL reaction as follows, with a 1:1 molar ratio of insert:vector. Incubate at 22℃ for 30 minutes

Reagent	Amount
linearized vector (100-200 ng)	x µl
PCR insert	y 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.

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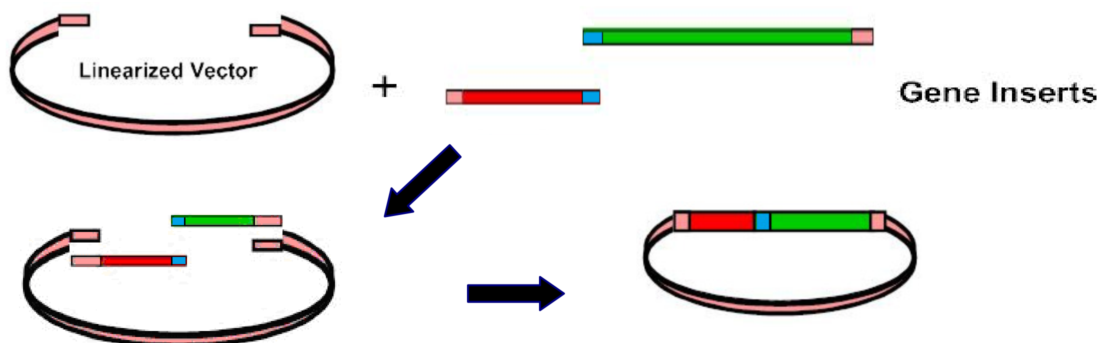
Insertion of multiple DNA fragments

The Quick PCR™ Cloning Kit allows for the insertion of multiple DNA fragments into the desired vector using only a single reaction.



1) Linearize the vector using either one or two restriction enzymes and purify the fragment, as above.

2) Design each of the gene-specific primers; for optimal results, 15-18 base pair of overlap among DNA fragments is recommended.

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

4) Isolate the PCR products by gel extraction and measure the concentrations of the PCR products.

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℃ 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**

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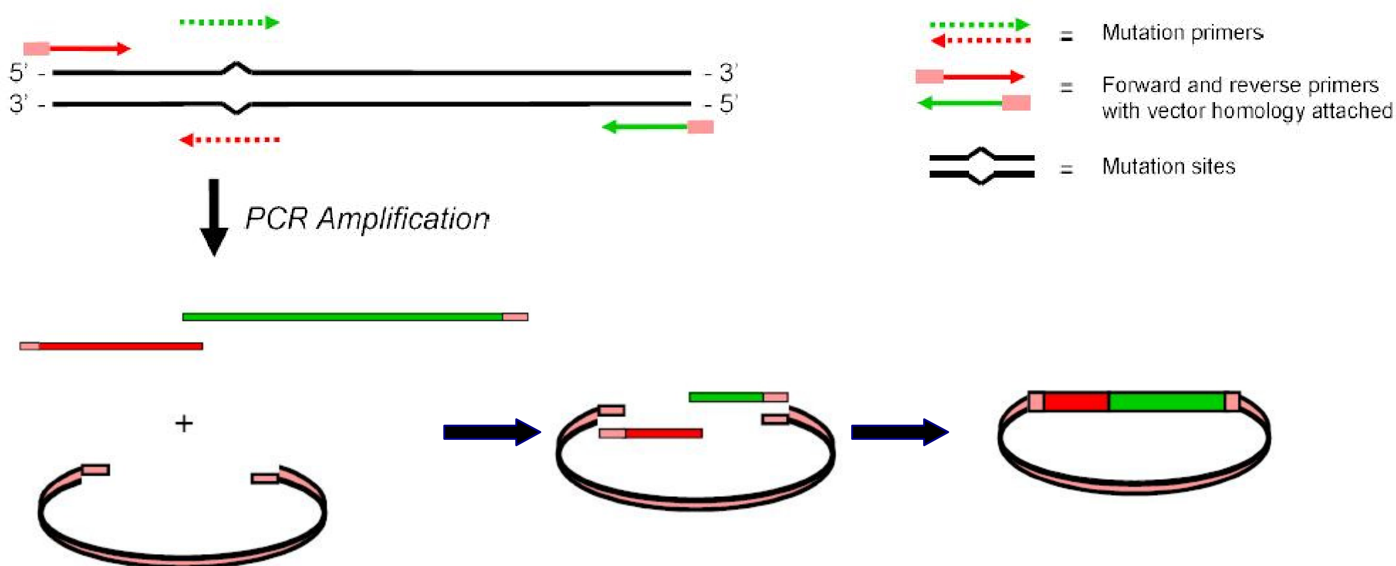
6) Add 1 µl Reagent B. Incubate microfuge tube on ice for 5 minutes.

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

Adding or "Fixing" mutations in DNA fragments

The Quick PCR™ Cloning 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.



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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.

Use of the Quick PCR™ Kit requires performance of the polymerase chain reaction (PCR), which is the subject of European Pat. Nos. 201,184 and 200,362, and U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 owned by Hoffmann-La Roche. Purchase of the Quick PCR™ kit does not include or provide a license with respect to these patents or any other PCR-related patent owned by Hoffmann-La Roche or others. Users of the Quick PCR™ kit may be required to obtain a license, depending on the country in which the system is used. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.

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