Change for the better.

Mandarin Ducks *(Aix galericulata)* are frequently featured in Chinese art and are regarded as a symbol of fidelity. The white mandarin's white plumage is caused by a sex-linked, recessive mutation, leading to a disorder in melanin deposition in the feathers, known as leucism.

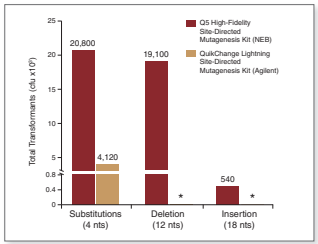
Q5® Site-Directed Mutagenesis Kit

The Q5 Site-Directed Mutagenesis Kit enables rapid, site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours. The kit utilizes Q5 Hot Start High-Fidelity DNA Polymerase, along with custom mutagenic primers to create substitutions, deletions and insertions in a wide variety of plasmids. Transformation into high-efficiency NEB 5-alpha Competent *E. coli* cells ensures robust results with plasmids up to, at least, 14 kb in length.

Advantages:

- Generation of mutations, insertions or deletions in plasmid DNA
- Non-overlapping primer design ensures robust, exponential amplification and generates a high % of desired mutations from a wide range of templates
- Intramolecular ligation and transformation into NEB 5-alpha results in high colony yield
- Low error rate of Q5 High-Fidelity DNA Polymerase reduces screening time
- Use of standard primers eliminates need for phosphorylated or purified oligos
- Easy-to-use master mix format available with or without competent cells

NEB's Q5 SDM Kit delivers higher transformation efficiency than Agilent's QuikChange® SDM Kit



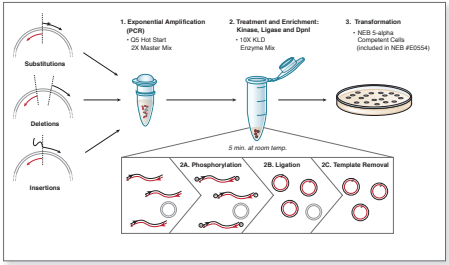
Results from a substitution reaction (4 nt) using the back-to-back Control SDM Primer Mix and Control SDM Plasmid (6.7 kb) are shown, along with results from a 12 nt deletion experiment (5.8 kb plasmid) and an 18 nt insertion experiment (7.0 kb plasmid). In all three cases, over 90% of the resultant colonies had incorporated the desired mutation(s). Results are normalized to total transformants if cells were not diluted prior to plating. For comparison, the same substitution reaction (4 nt) was performed with the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent) following Agilent's protocol and using Agilent's primer design tool to design overlapping primers.

**Note that the QuikChange kit does not accommodate deletions and insertions of this size, so no comparison could be made for these experiments.*

NEW ENGLAND BioLabs Inc.

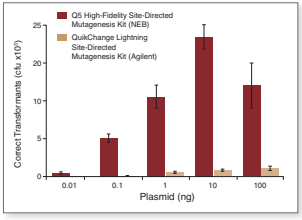
Q5 Site-Directed Mutagenesis Kit

Q5 Site-Directed Mutagenesis Kit Overview



This kit is designed for rapid and efficient incorporation of insertions, deletions and substitutions into double-stranded plasmid DNA. The first step is an exponential amplification using standard primers and a master mix formulation of Q5 Hot Start High-Fidelity DNA Polymerase. The second step involves incubation with a unique enzyme mix containing a kinase, a ligase and DpnI. Together, these enzymes allow for rapid circularization of the PCR product and removal of the template DNA. The last step is a high-efficiency transformation into chemically-competent cells (included in NEB #E0554).

NEB's Q5 SDM Kit produces a higher yield of correct transformants than the Agilent QuikChange Kit



The substitution reaction (4 nt) using the back-to-back Control SDM Primer Mix and Control SDM Plasmid (6.7 kb) was performed with various amounts of plasmid template as indicated. For comparison, the same experiment was performed with the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent) following Agilent's protocol and using Agilent's primer design tool to design overlapping primers. Results are normalized to total transformants if cells were not diluted prior to plating. Although successful experiments are possible outside this range, we recommend using between 0.1 and 100 ng of plasmid to achieve robust results.

Ordering Information

PRODUCT	NEB #	SIZE
Q5 Site-Directed Mutagenesis Kit	E0554S	10 reactions
Q5 Site-Directed Mutagenesis Kit (Without Competent Cells	E0552S	10 reactions
COMPANION PRODUCT		
NEB 5-alpha Competent *E. coli* (High Efficiency)	C2987I/H	6 x 0.2 ml/20 x 0.05 ml
SOC Outgrowth Medium	B9020S	4 x 25 ml

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