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Q5® Site-Directed Mutagenesis (E0552) V.4

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The Q5 Site-Directed Mutagenesis Kit (Without Competent Cells) enables rapid, site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours (Figure 1). The kit utilizes the robust Q5 Hot Start High-Fidelity DNA Polymerase along with custom mutagenic primers to create insertions, deletions and substitutions in a wide variety of plasmids. After PCR, the amplified material is added directly to a unique Kinase-Ligase-DpnI (KLD) enzyme mix for rapid (5 minutes), room temperature circularization and template removal (Figure 2). Transformation into high-efficiency chemically-competent *E. coli*, not supplied, ensures robust results with plasmids up to at least 20 kb in length. Kit is available with competent cells (NEB #E0554).

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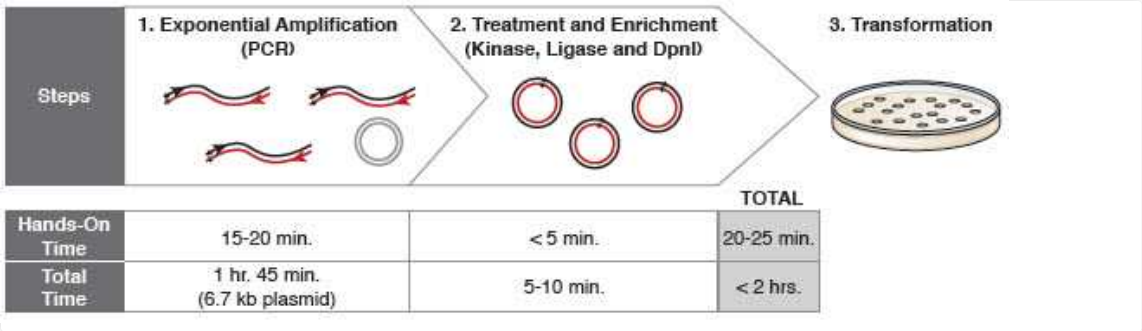
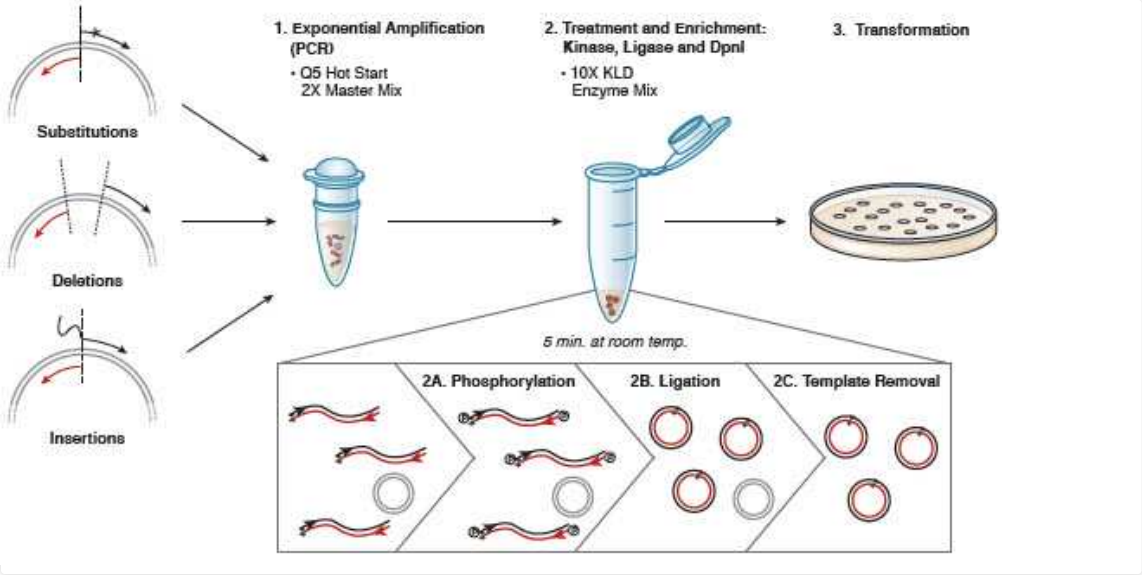
Figure 1: Site-specific mutagenesis proceeds in less than 2 hours.



The use of a master mix, a unique multi-enzyme KLD enzyme mix, and a fast polymerase ensures that, for most plasmids, the mutagenesis reaction is complete in less than two hours.

Figure 2: Q5 Site-Directed Mutagenesis Overview.



This kit is designed for rapid and efficient incorporation of insertions, deletions and substitutions into doublestranded plasmid DNA. The first step is an exponential amplification using standard primers and a master mix fomulation 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 chemicallycompetent cells (not provided).

Figure 3: Primer Design for Q5 Site-Directed Mutagenesis

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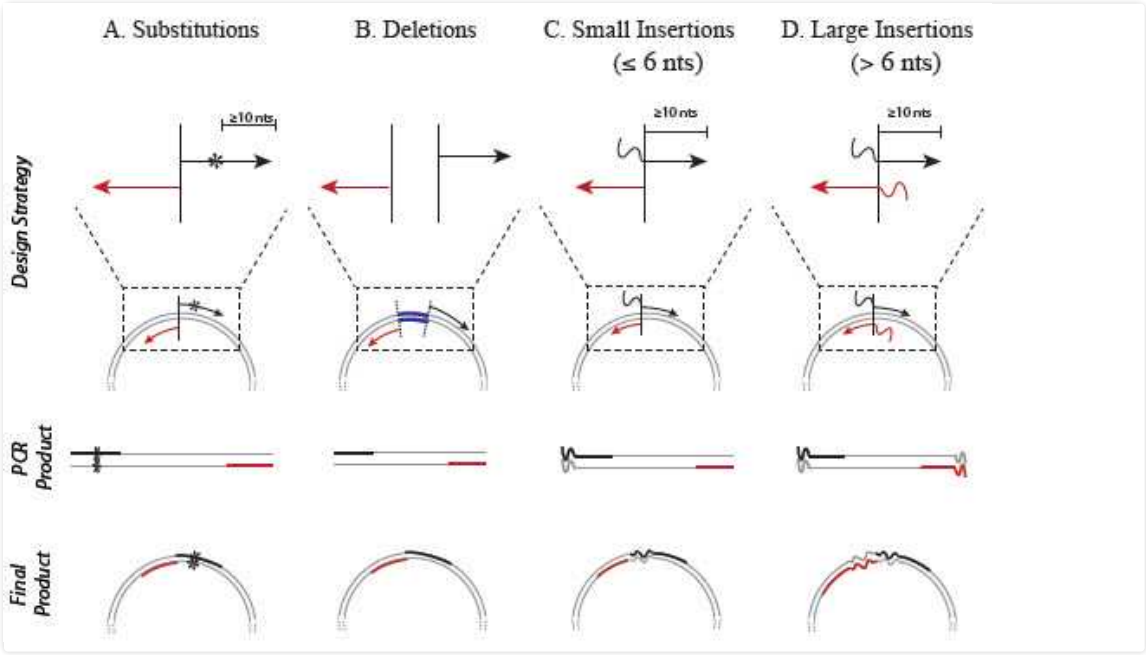
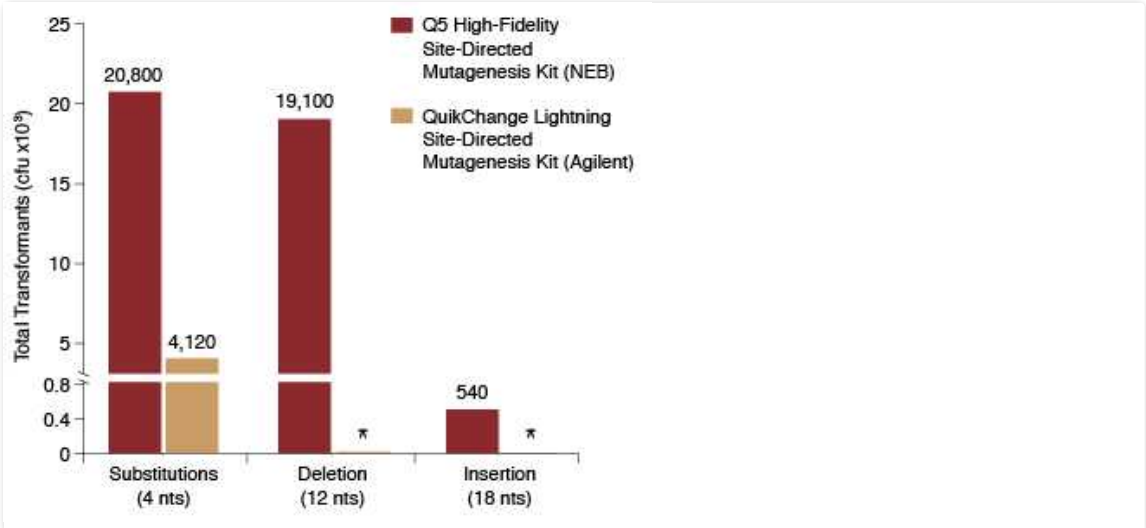


Substitutions, deletions and insertions are incorporated into plasmid DNA through the use of specifically designed forward (black) and reverse (red) primers.**Unlike kits that rely on linear amplification, primers designed for the Q5 Site-Directed Mutagenesis Kit should not overlap to ensure that the benefits of exponential amplification are realized.**A) Substitutions are created by incorporating the desired nucleotide change(s) (denoted by *) in the center of the forward primer, including at least 10 complementary nucleotides on the 3´side of the mutation(s). The reverse primer is designed so that the 5´ends of the two primers anneal backto-back. B) Deletions are engineered by designing standard, non-mutagenic forward and reverse primers that flank the region to be deleted. C) Insertions less than or equal to 6 nucleotides are incorporated into the 5´ end of the forward primer while the reverse primer anneals back-to-back with the 5´ end of the complementary region of the forward primer. D) Larger insertions can be created by incorporating half of the desired insertion into the 5´ ends of both primers. The maximum size of the insertion is largely dictated by oligonucleotide synthesis limitations.

Figure 4: NEB's Q5 SDM Kit delivers higher transformation efficiency than Agilent's QuikChange®SDM Kit



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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.

MATERIALS

Q5 Site-Directed Mutagenesis Kit (Without Competent Cells) - 10 rxns **New England Biolabs Catalog #E0552S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Exponential Amplification (PCR)

1

Assemble the following reagents in a thin-walled PCR tube:

A	B	C
	25 µl RXN	**FINAL CONC.**
Q5 Hot Start High-Fidelity 2X Master Mix	12.5 µl	1X
10 µM Forward Primer	1.25 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	0.5 µM
Template DNA (1–25 ng/µl)	1 µl	1-25 ng
Nuclease-free water	9.0 µl	

2

Mix reagents completely.

3

Transfer to a thermocycler and perform the following cycling conditions:
Thermocycling Conditions for a Routine PCR:

A	B	C
STEP	**TEMP**	**TIME**
Initial Denaturation	98°C	30 seconds
25 Cycles	98°C	10 seconds
	50–72°C*	10–30 seconds
	72°C	20–30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4–10°C	

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* For a Q5-optimized annealing temperature of mutagenic primers, please use NEBaseChanger™, the online NEB primer design software. For pre-designed, back-to-back primer sets, a Ta = Tm + 3 rule can be applied, but optimization may be necessary.

Kinase, Ligase & DpnI (KLD) Treatment

4 

Assemble the following reagents:

A	B	C
	VOLUME	**FINAL CONC.**
PCR Product	1 µl	
2X KLD Reaction Buffer	5 µl	1X
10X KLD Enzyme Mix	1 µl	1X
Nuclease-free Water	3 µl	

5 

Mix well by pipetting up and down.

6 

Incubate at **Room temperature** for **00:05:00** .

Transformation

7 Thaw **50 µL aliquot of chemically competent E. coli cells** **On ice** .

NEB 5-alpha Competent *E. coli* (High Efficiency), NEB #C2987, are recommended

8 

Add **5 µL KLD mix** from the "KLD Section" above to the tube of thawed cells.

9 

Carefully flick the tube 4-5 times to mix. **Do not vortex.**

10 

Place the mixture **On ice** for **00:30:00** .

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11 Heat shock at **42 °C** for **00:00:30** .

12 Place **On ice** for **00:05:00** .

13 

Pipette **950 µL room temperature SOC** into the mixture.

14 

Incubate at **37 °C** for **01:00:00** with shaking (**250 rpm**).

15 

Mix the cells thoroughly by flicking the tube and inverting.

16 

Spread **50 µL** - **100 µL** onto a selection plate.

17 

Incubate **Overnight** at **37 °C** .

It may be necessary (particularly for simple substitution and deletion experiments) to make a 10- to 40-fold dilution of the transformation mix in SOC prior to plating, to avoid a lawn of colonies.

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