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Q5® Site-Directed Mutagenesis (E0552) v.3

New England Biolabs[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.beurjev6

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ABSTRACT

This protocol defines methods for the Q5® Site-Directed Mutagenesis Kit without competent cells.

EXTERNAL LINK

https://www.neb.com/protocols/2014/03/21/q5-site-directed-mutagenesis-kit-without-competent-cells-protocol-e0552

MATERIALS

NAME	CATALOG #	VENDOR
Q5 Site-Directed Mutagenesis Kit (Without Competent Cells) - 10 rxns	E0552S	New England Biolabs

SAFETY WARNINGS

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:

	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.

Citation: New England Biolabs (04/09/2020). Q5ÃÂ® Site-Directed Mutagenesis (E0552). https://dx.doi.org/10.17504/protocols.io.beurjev6

3

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

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	

* 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:

	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

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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** .

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 50-100 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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