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

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Protocol status: Working
We use this protocol and it's working

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Abstract

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

Materials

MATERIALS

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

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.

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 Cycle	98°C	10 seco

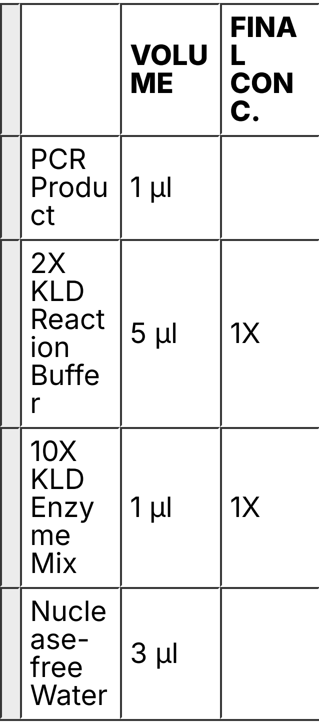
	s		nds
		50–72°C *	10–30 seconds
		72°C	20–30 seconds/kb
	Final Extension	72°C	2 minutes
	Hold	4–10°C	

Note

* 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 .

Note

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 .

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 .

Note

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.