



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

New England Biolabs¹

Apr 09, 2020

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dx.doi.org/10.17504/protocols.io.beurjev6







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 V CATALOG # V VENDOR V

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	12.5 μΙ	1X
Mix		
10 μM Forward Primer	1.25 μΙ	0.5 μM
10 μM Reverse Primer	1.25 μΙ	0.5 μM
Template DNA (1-25 ng/μl)	1 μΙ	1-25 ng
Nuclease-free water	9.0 µl	

2



Mix reagents completely.

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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



Assemble the following reagents:

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

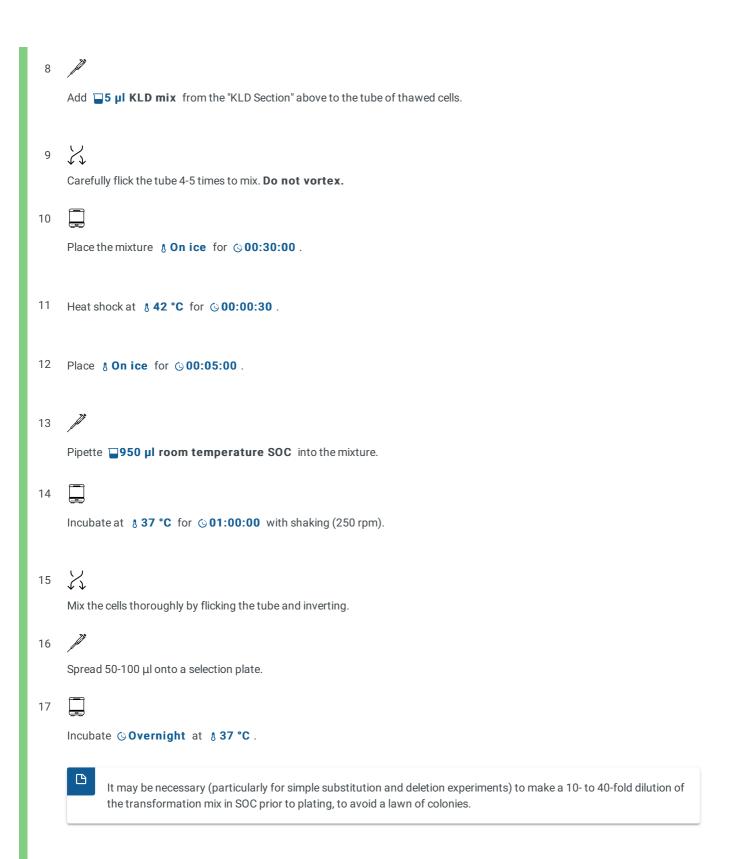
Mix well by pipetting up and down.

Incubate at & Room temperature for © 00:05:00.

Transformation



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



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