Q5® Site-Directed Mutagenesis Kit Quick Protocol (E0554)

New England Biolabs

Abstract

This is the quick protocol for the Q5® Site-Directed Mutagenesis Kit (E0554)

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Before start

Primers should be designed with 5´ ends annealing back-to-back. We recommend using the NEB online design software, NEBaseChanger™.

Protocol

Exponential Amplification (PCR)

Step 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 μΜ
10 μM Reverse Primer	1.25 µl	0.5 μΜ
Template DNA (1-25 ng/µl)	1 μΙ	1-25 ng
Nuclease-free water	9.0 µl	



. E0552 Q5 PCR Mixture

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Step 1.1.

Q5 Hot Start High-Fidelity 2X Master Mix 12.5 ul

Step 1.2.

10 μM Forward Primer **1.25ul**

Step 1.3.

10 μM Reverse Primer **1.25ul**

Step 1.4.

Template DNA (1-25 ng/µl) 1ul

Step 1.5.

Nuclease-free water 9ul

Exponential Amplification (PCR)

Step 2.

Mix reagents completely.

Exponential Amplification (PCR)

Step 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	
	98°C	10 seconds	
25 Cycles	50-72°C*	10-30 seconds	
	72°C	20-30 seconds/kb	
Final Extension	72°C	2 minutes	
Hold	4-10°C		

NOTES

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For mutagenic primers, please use the Ta provided by the online NEB primer design software, $NEBaseChanger^{TM}$.

Kinase, Ligase & DpnI (KLD) Treatment

Step 4.

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 μΙ	



. E0552 KLD Mixure

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Step 4.1.

PCR Product 1ul

Step 4.2.

2X KLD Reaction Buffer 5ul

Step 4.3.

10X KLD Enzyme Mix 1ul

Step 4.4.

Nuclease-free Water 3ul

Kinase, Ligase & DpnI (KLD) Treatment

Step 5.

Mix well by pipetting up and down.

Kinase, Ligase & DpnI (KLD) Treatment

Step 6.

Incubate at room temperature for 5 minutes.

O DURATION

00:05:00

Transformation

Step 7.

Add **5 µl** of the KLD mix from the "KLD Section" above to 50 µl of chemically-competent cells.

Transformation

Step 8.

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

Transformation

Step 9.

Place the mixture on ice for 30 minutes.

© DURATION

00:30:00

Transformation

Step 10.

Heat shock at 42°C for 30 seconds.

O DURATION

00:00:30

Transformation

Step 11.

Place on ice for 5 minutes.

© DURATION

00:05:00

Transformation

Step 12.

Pipette 950 µl of room temperature SOC into the mixture.



950 µl Additional info:



SOC Outgrowth Medium - 100 ml B9020S by New England Biolabs

Transformation

Step 13.

Incubate at 37°C for 60 minutes with shaking (250 rpm).

© DURATION

01:00:00

Transformation

Step 14.

Mix the cells thoroughly by flicking the tube and inverting.

Transformation

Step 15.

Spread 40-100 µl onto appropriate selection plate

Transformation

Step 16.

Incubate overnight at 37°C

© DURATION 15:00:00