Q5® Site-Directed Mutagenesis (E0552)

New England Biolabs

Abstract

This is the protocol for the Q5® Site-Directed Mutagenesis Kit without competent cells

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Materials

NEB 5-alpha Competent E.coli (High Efficiency) - 6x0.2 ml C2987I by New England Biolabs

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

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 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	

PROTOCOL

. E0552 Q5 PCR Mixture

CONTACT: New England Biolabs
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
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	

ANNOTATIONS

New England Biolabs 09 Oct 2014
* 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

Step 4.
Assemble the following reagents:

PROTOCOL

. 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.

DURATION
00:05:00

Transformation

Step 7.
Thaw a **50 µl** aliquot of chemically competent E. coli cells on ice.

ANNOTATIONS
New England Biolabs 26 Jan 2015
NEB 5-alpha Competent E. coli (High Efficiency), NEB #C2987, are recommended

Transformation

Step 8.
Add **5 µl** of the KLD mix from the "KLD Section" above to the tube of thawed cells.

Transformation

Step 9.
Carefully flick the tube 4-5 times to mix. **Do not vortex.**

Transformation

Step 10.
Place the mixture on ice for 30 minutes.

DURATION
00:30:00

Transformation

Step 11.
Heat shock at 42°C for 30 seconds.

DURATION
00:00:30

Transformation

Step 12.
Place on ice for 5 minutes.

DURATION
00:05:00

Transformation

Step 13.
Pipette 950 µl of room temperature SOC into the mixture.

AMOUNT
950 µl Additional info:

REAGENTS
SOC Outgrowth Medium - 100 ml B9020S by New England Biolabs

Transformation

Step 14.
Incubate at 37°C for 60 minutes with shaking (250 rpm).

DURATION
01:00:00

Transformation

Step 15.
Mix the cells thoroughly by flicking the tube and inverting.

Transformation

Step 16.

Spread 50-100 µl onto a selection plate.

Transformation

Step 17.

Incubate overnight at 37°C

DURATION

15:00:00

NOTES

New England Biolabs 03 Oct 2014

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