Site Directed Mutagenesis

Introduction

The Q5 Site-Directed Mutagenesis Kit enables rapid, site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours (Figure 1). The kit utilizes the robust Q5 Hot Start High-Fidelity DNA Polymerase along with custom mutagenic primers to create insertions, deletions and substitutions in a wide variety of plasmids. After PCR, the amplified material is added directly to a unique Kinase-Ligase-DpnI (KLD) enzyme mix for rapid (5 minutes), room temperature circularization and template removal Transformation into high-efficiency NEB 5-alpha Competent E. coli, provided with the kit, ensures robust results with plasmids up to at least 14 kb in length.

Materials

- Q5 Hot Start High-Fidelity 2X Master Mix (2X)
- KLD Enzyme Mix (10X)
- KLD Reaction Buffer (2X)
- Control SDM Primer Mix (10 µM each)
- Control SDM Plasmid (5 µg/ml)
- NEB 5-alpha Competent E. coli (High Efficiency)
- pUC19 Transformation Control Plasmid (50 pg/µl)
- SOC Outgrowth Medium

Procedure

Step I: Exponential Amplification

1. Assemble the following reagents in a thin-walled PCR tube.

Table1

	A	B	C
1		25 µl RXN	FINAL CONC
2	Q5 Hot Start High-Fidelity 2X Master Mix	12.5 µl	1X
3	10 µM Forward Primer	1.25 µl	0.5 µM
4	10 µM Reverse Primer	1.25 µl	0.5 µM
5	Template DNA (1–25 ng/µl)	1 µl	1-25 ng
6	Nuclease-free water	9.0 µl	

2. Mix reagents completely, then transfer to a thermocycler.

3. Perform the following cycling conditions:

Table2

	A	B	C
1	STEP	TEMP	TIME
2	Initial Denaturation	98°C	30 seconds
3	25 Cycles	98°C	10 seconds
4		50–72°C*	10–30 seconds
5		72°C	20–30 seconds/kb
6	Final Extension	72°C	2 minutes
7	Hold	4–10°C	

Step II: Kinase, Ligase & DpnI (KLD) Treatment

4. Assemble the following reagents:

Table3

	A	B	C
1		Volume	Final Conc
2	PCR Product	1 µl	
3	2X KLD Reaction Buffer	5 µl	1X
4	10X KLD Enzyme Mix	1 µl	1X
5	Nuclease-free Water	3 µl	

5. Mix well by pipetting up and down and incubate at room temperature for 5 minutes.

Step III: Transformation

6. Thaw a tube of NEB 5-alpha Competent E. coli cells on ice.
7. Add 5 µl of the KLD mix from Step II to the tube of thawed cells. Carefully flick the tube 4-5 times to mix. Do not vortex.
8. Place the mixture on ice for 30 minutes.
9. Heat shock at 42°C for 30 seconds.
10. Place on ice for 5 minutes.
11. Pipette 950 µl of room temperature SOC into the mixture.
12. Incubate at 37°C for 60 minutes with shaking (250 rpm).
13. Mix the cells thoroughly by flicking the tube and inverting, then spread 50-100 µl onto a selection plate and 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.