Site Directed Mutagenesis

Introduction

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Procedures List

Q5® Site-Directed Mutagenesis Kit Protocol

Product Manual

Materials

- Q5 Site-Directed Mutagenesis Kit
  - NEB® 5-alpha Competent *E. coli* (High Efficiency)
  - Q5®-Hot Start High-Fidelity 2X Master Mix
  - SOC Outgrowth Medium
  - Control SDM Plasmid
  - Control SDM Primer Mix
  - KLD Enzyme Mix
  - KLD Reaction Buffer
  - pUC19 Vector
- Forward and Reverse Primers
- Template DNA
- Nuclease-free water
- Ice
- Selection Plates

Procedure

Step I: Exponential Amplification (PCR)

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

Table1

	A	B	C
1		**25 µl RXN**	**Final Concentration**
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:

   Thermocycling Conditions for a Routine PCR:
   ** 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.*

Table2

	A	B	C
1	**Step**	**Temperature °C**	**Time**
2	**Initial Denaturation**	98	30 seconds
3	**25 Cycles**	98	10 seconds
4		50-72	10-30 seconds
5		72	20-30 seconds/kb
6	**Final Extension**	72	2 minutes
7	**Hold**	4-10	

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

4. Assemble the following reagents:

Table3

	A	B	C
1		**Volume**	**Final Concentration**
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