NEBaseChanger Summary - Mon May 15 2023

Input sequence:BRho-GFPII-1D4

Type of mutagenesis: substitution

Mutagenesis region: 389 to 389

Replace/insert: A

Result

```
   N  Y  F  L  L  L  W  T  T  R  L  N  R  Q
  *  L  F  S  S  A  M  D  N  S  S  *  P  S  K
 V  I  I  F  F  C  Y  G  Q  L  V  L  T  V  K
GTAATTATTTTCTTCTGCTATGGACAACTCGTCTTaACCGTCAAAG
CATTAATAAAAGAAGACGATACCTGTTGAGCAGAATTGGCAGTTTC
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Required Primers

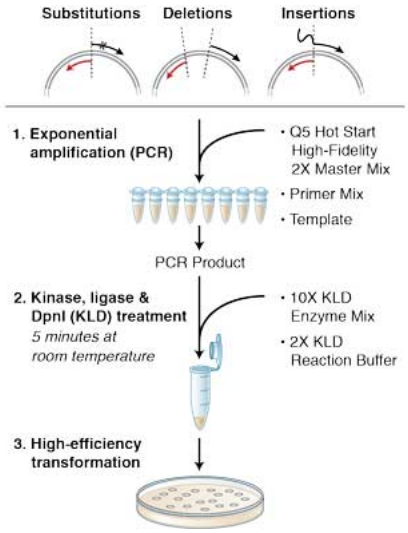
Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
Q5SDM_5/16/2023_F	AACTCGTCTTaACCGTCAAAG	21	43	60°C	**61°C**
Q5SDM_5/16/2023_R	GTCCATAGCAGAAGAAAATAATTAC	25	32	60°C	

* Ta (recommended annealing temperature)

PROTOCOL

The full Q5 Site-Directed Mutagenesis Protocol can be found in the manual, which can be downloaded from: www.NEB.com/CN/E0554

For your convenience, a quick protocol is presented here:



Step I: Exponential Amplification (PCR)

	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	

Cycling Conditions:

STEP	TEMP	TIME
Initial Denaturation	98 °C	30 seconds
	98 °C	10 sec
25 Cycles	61 °C*	10-30 sec
	72 °C	20-30 seconds/kb
Final Extension	72 °C	2 minutes
Hold	4-10 °C	∞

*The recommended annealing temperature of 61 °C is specific for the mutagenic primers listed above when amplified with Q5 DNA Polymerase.

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

	10 µl RXN	FINAL CONC.

PCR Product	1 µl	
2X KLD Reaction Buffer	5 µl	1X
10X KLD Enzyme Mix	1 µl	1X
Nuclease-free water	3 µl	

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

Step III: Transformation

1. Add 5 µl of the KLD reaction mixture directly from step II to 50 µl of chemically-competent cells.
2. Incubate on ice for 30 minutes.
3. Heat shock at 42 °C for 30 seconds.
4. Incubate on ice for 5 minutes.
5. Add 950 µl of SOC, gently shake at 37 °C for 1 hour.
6. Spread 40-100 µl onto appropriate selection plate and incubate overnight at 37 °C (if necessary, make a 10-100 fold dilution of recovered cells before plating to avoid a lawn of colonies).

Sequence after Q5 SDM

TTCACGCTTATTTTGTCTTTGGGCCGACAGGGTGCAACCTCGAAGGGTTCTTCGCAACACTT
GGAGGAGAGATCGCACTCTGGTCTCTTGTAGTACTTGCTATAGAGAGGTATGTGGTAGTGTG
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GGGATGCAATGCAGTTGTGGAATCGATTATTATACTTCTAGGCAAGAGGTAAACAACGAGTC
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