Protocol for Site-Directed Mutagenesis

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1; Plasmid preparation:

Gene in pMOLuc (or pMOKan) with target site for mutations.

2; Mutant strand synthesis;

Preparation PCR reaction Mix: (Per 50 ul reaction)

5x HF Buffer (NEB)	10ui
dNTPs (10mM each)	3ul
DMSO	2.5ul
$MgCl_2$ (50mM)	2.5ul
Primer #1 (330ng/ul)	1ul
Primer #2 (330ng/ul)	1ul
ddH_2O	q.s to 50ul
Phusion DNA polymerase (NEB)	0.5ul
Template DNA (plasmid)	(50-200ng)
	50.0ul

50ul sample is divided into two PCR reaction tube for PCR amplification.

Perform thermal cycling to;

96°C X2’ X 1 cycle

92°C X 20"
55°C X 30" X 15 cycles (up to 20 cycles)
70°C X 4’ (or **2-4kb/min**)

70°C X 5′ X 1 cycle

3: PC-8 extraction of DNA samples.

4: Ethanol precipitation of DNA samples. Dissolv DNA pellet into 84ul ddH_2O

5: Digest parental methylated and hemmethylated DNA with Dpn I enzyme,

Reaction Mix: (Per 100ul reaction)

10 X buffer	10ul
BSA	2 ul
DNA sample and ddH_2O	84ul
Dpn I	4 ul
	100 ul

At 37°C for 30 min.

6: Ethanol precipitation of DNA samples, Dissolv DNA pellet into 30ul ddH_2O

7: Transformation:

Take 5-10ul DNA sample into electrocompetent DH10B bacterial cells, Perform electroporation at 1.8KV, followed by adding 500ul LB to the cuvette,
Mix bacterial cells, immediately plate and incubate the remaining cell, at 37^0C water bath for 20-30min, followed by plating the mix, Incubate at 37^0C incubator overnight.