In Vitro Mutagenesis

I. General Procedure

1. Turn on Robocycler to warm it up.

2. Thaw all necessary components (on ice)

3. Dilute template DNA to 5ng/µl (5µg/ml).

4. Label PCR tubes.

5. Aliquot complimentary primers to respective PCR tubes. Place on ice.

6. Prepare necessary volume of reaction cocktail. Do this on ice.

7. Add reaction cocktail to tubes with complimentary primers.

8. Place in Robocycler and Start PCR process.

II. Reaction Conditions:

	Vol to use	Final Rxn []	Rxn Cocktail (for 5 rxns)
DNA template	5.0µl	(25ng)	25.0µl
10X Reaction Buffer	5.0µl	(1X)	25.0µl
dNTP mix	1.0µl		5.0µl
DMSO	2.5µl	5%	12.5µl
ddH_2O (to final vol of 50µl)	33.0		165.0
PfuTurbo Polymerase	1.0µl	2.5U	5.0µl
Oligo #1	1.25µl	125ng	-
Oligo #2	1.25µl	125ng	-

(No need to overlay sample with mineral oil when using the Robocycler fitted with the Hot Top)
(Primers are assumed to be at 100ng/µl)

III. PCR Conditions (for a 4.5kb plasmid):

Segment	Cycles	Temperature	Duration
1	1	95°C	60 sec
2	18	95°C	30 sec
		55°C	60 sec
		68°C	9.0 min (2min/kb plasmid)
3	1	6°C	∞

IV. *Dpn*I Digestions:

PCR Product	50µl
*Dpn*I enzyme	1µl
37°C	~1h

Remove approximately 10% of the digest reaction to a second microfuge tube
Add 2µl loading dye
Run on gel
Transform 5µl of PCR product into 50µl of $CaCl_2$ competent *E. coli*DH5α
Store remainder of PCR reaction product at –20°C.