Mutagenesis Using FailSafe Buffers with Stratagene Kit

1) Use the FailSafe PCR 2X PreMixes which produced the correct size fragments during the Selection Procedure. Set up the following rxn, on ice:
   X µL plasmid template (30 ng)
   X µL primer 1 (125 ng)
   X µL primer 2 (125 ng)
   1 µL Epicentre FailSafe PCR Enzyme Mix
   X µL to sterile water (to 25 µL)
   Add:
   25µL FailSafe PCR 2X PreMix (A-L, use one that worked)
2) Run samples in the thermocycler according to the following parameters:

95°C – 30s		1 cycle
16 cycles:	95°C – 30s	
	55°C – 1 min	
	68°C – 1min/kb of plasmid length	
4°C		hold

3) Add 1µL *DpnI* restriction enzyme (Stratagene Kit, -20°C) directly to each PCR rxn. (*DpnI* digests methylated DNA, leaving the newly synthesized mutated DNA untouched.) Mix gently. Incubate at 37°C for 1 hour.
4) Thaw XL-1 Blue Supercompetent cells (Stratagene Kit, -80°C) on ice. Aliquot 50µL of cells into pre-chilled PCR tubes.
5) Transfer 1 µL of DpnI-treated DNA from each PCR reaction to an aliquot of cells. Stir with pipette tip.
6) Incubate on ice for 30 min.
7) Heat pulse the transformation rxns for 45s at 42°C (use the thermocycler). Incubate on ice for 2 min.
8) To culture tubes, add 0.5 mL of S.O.C. Medium (pre-warmed to 37°C). Pipette transformed cells into the media. Shake for 1 hour at 225 rpm.
9) Plate 200 µL of each transformation reaction onto plates. Incubate O/N at 37°C.