Site Directed Mutagenesis with Stratagene Pfu Turbo

Procedure:
PCR mixture# 50 µl:
33.5 µl ddH2O
5 µl 10X Pfu Turbo Reaction mix
2 µl 2.5 mM dNTP
2.5 µl DMSO
2 µl Primer mix (10 µM each)* (final 0.4 µM each)
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2 µl Template DNA (10 pg-200 ng)
1 µl Pfu turbo DNA Polymerase

PCR Reaction:
Step 1: Heating 95˚C for 2 min
Step 2: Heating 95˚C for 20s
Step 3: Anealing 55˚C for 30s
Step 4: Elongation 68˚C for 14 min
Step 5: Repeat Steps 2-4 for 19 cycles
Step 6: Elongation 68˚C for 10 min
Step 7: 4˚C forever
Step 8: End

After PCR, Transfer 30 µl to a new tube. incubate each sample with 1µl of Dpn I for 1 hr at 37˚C. At the mean time, take 5 µl from the rest of PCR tube to run on agarose gel.

Take 2 µl of DpnI digested mixture for transformation.

Note:
Perform a control reaction without primers, do PCR and DpnI digestion as the same.