Multiple mutations (using the stratagene multi kit):

1. 10x buf: 2.5
2. DNA(template, miniprep): 1, 1(1/2), 1(1/5), 1(1/10)
3. primer1 (100ng/ul): 1
4. primer2 (100ng/ul): 1
5. primer3 (100ng/ul): 1
6. dntp_mix: 1
7. Enzyme: 1
8. H2O: add until total 25 ul =16.5

Mix

Mix 10x buf, primer1,2,3, H2O together, for 5 groups: 2.5X5=12.5, 1X5=5, 1X5=5, 1X5=5, 16.5X5=82.5, aliquote 2.5+16.5+1+1+1=22 to each tube, add DNA, DNTP, Enzyme, 1, 1,1.

Run Prset_multi

1. cycle 1, 95oC, 1 min
2. cycle 30, 95oC, 1min
   - 55oC, 1min
   - 65oC, 12 min

group 4 gives the best results.