1-primer mutagenesis

Primer design

Design one forward primer to anneal at 55°C both upstream and downstream of the point mutation (approximately 45bp total primer length). Use the primer design spreadsheet at http://macrolab.berkeley.edu if you need it. Change the fewest number of bases possible to get the desired amino acid.

Mutagenesis reaction

400ng plasmid (~2uL)
2uL of 5uM primer
0.4uL 100mM dNTPs
1uL PfuTurbo
5uL Pfu buffer
ddH_2O to 50uL total volume

Note: if you order your oligo from a commercial source, it was already run over a desalting column. No further purification is necessary.

95° – 3min
    95° – 30s
    53° – 1min 18 cycles
    68° – 2min/kb
68° – 4min/kb

DpnI digest

4uL mutagenesis reaction
2uL NEB buffer 4
0.5uL DpnI
13.5uL ddH_2O

Incubate at 37C for 2-2.5 hours

Transform cloning strain of E. coli (e.g. XL-1 Blues) with 6uL of DpnI rxn (30min on ice, 45s heat shock at 42C, 2 min on ice, add 500uL 2YT and plate 200uL on appropriate antibiotic plate).

I get ~400cols/plate, ~45% mutants and 55% wild type plasmids using this protocol.

Things NOT to do:
Don't use Herculase II
Don't undercut the extension time
Don't use any buffer other than the commercially supplied one