Quick Change Mutagenesis with Pfu Pol (older method)

Hahn Lab 2001

This method uses the two-stage PCR protocol allowing introduction of multiple mutations, deletions and insertions (Wang and Malcolm, bioTechniques 26:680-682 (1999). We almost always use the KOD extreme method instead of this older method.

1. Set up two separate primer extension reactions (one for each top and bottom primer) containing:

5 microliters 10X Pfu Buffer (supplied with enzyme)
1 microliter 10 micromolar primer (0.13 microgram 45-mer)
0.1 – 0.2 microgram plasmid template
1 microliter 10 mM dNTP mix
H2O to a final volume of 50 microliters

Add 1 microliter Pfu turbo polymerase (Stratagene)

Incubate:

1. 94 deg, 30 sec
2. 95 deg, 30 sec
3. 55 deg, 1 min
4. 68 deg, 2 min/kb up to 10 KB plasmid

Repeat steps 2-4 for a total of 4 cycles
Hold at 4 deg.

Combine 25 microliters from each extension reaction above. Add 1 microliter Pfu polymerase. Incubate as above, except repeat a total of 18 cycles.

Remove 25 microliters of the reaction. Add 10 units of DpnI enzyme. Mix well and incubate at 37 deg for at least one hour.

Transform 1 microliter of the reaction to electrocompetent cells. Plate 100 and 20 microliters of cells on separate plates. Expect ~100 colonies on the 20 microliter cell plate.
Notes:

Expected mutagenesis frequency about 70%.

Stratagene recommends gel purifying the primers for the highest mutagenesis frequency, but not routinely done.

Primer pairs should have similar annealing temperatures. Melting temperatures between 55-80 deg are suggested.