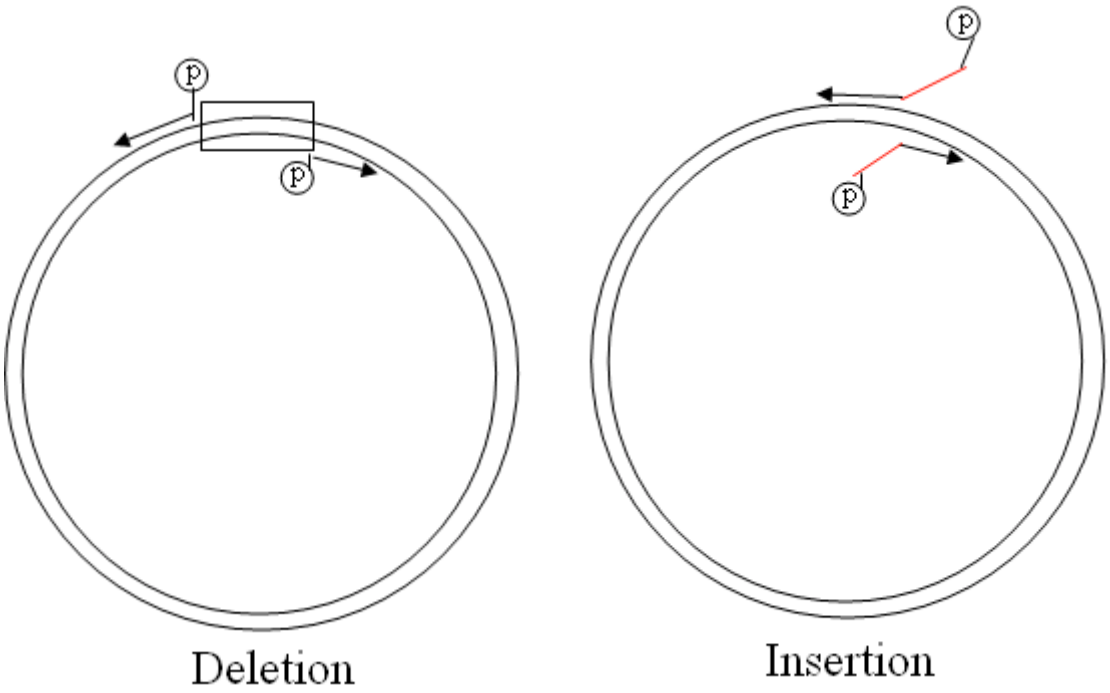
Deletion or Insertion of amino acids without the involvement of restriction enzyme sites

- Design primers as indicated:



Run PCR as following (strategene mutagenesis kit):

10x buffer:	2.5 ul
DNA:	1 ul (prediluted 1:5)
Primer1:	0.625 ul (stock solution 100ng/ul)
Primer2:	0.625 ul (stock solution 100ng/ul)
H2O:	19.25 ul
DNTP:	0.5 ul
Pfu Turbo:	0.5 ul

Digest parental DNA with 0.5 ul DPNi for >2hr 37oC.

Gel purify the PCR product, do blunt ligation:

5x buffer:	4 ul
DNA:	5 ul

Ligase:	1 ul
H2O:	10 ul

Do transformation

Deletion and Addition Inserts

Deletion and Addition using Site-direct mutagenesis Kit from Strategene

1. 10x buf: 2.5
2. DNA (miniprep): 1, 1(1/5), 1(1/10).
3. primer1 (100ng/ul): 0.625
4. primre 2 (100ng/u): 0.625
5. H2O: 19.25
6. dNTP: 0.5
7. pfu Turbo: 0.5

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mix 4 groups: 10xbuf, primer1, primer2, H2O: 2.5X4=10, 0.625X4=2.5, 0.625X4=2.5, 19.25X4=77, aliquote 2.5+0.625+0.625+19.25=23 to each tube. Add Dntp mix, DNA, pfu Turbo: 0.5, 1, 0.5

Run program Prset:

1. cycle 1, 95oC, 30 sec
2. cycle 18, 95oC, 30 sec
   - 55oC, 1min
   - 68oC, 12 min

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group 2 gives the best results.