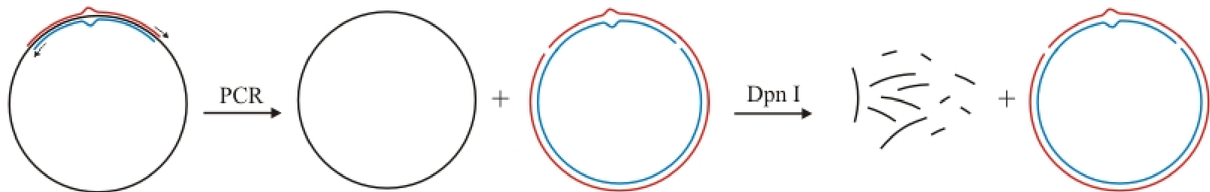
Introduction of point mutations

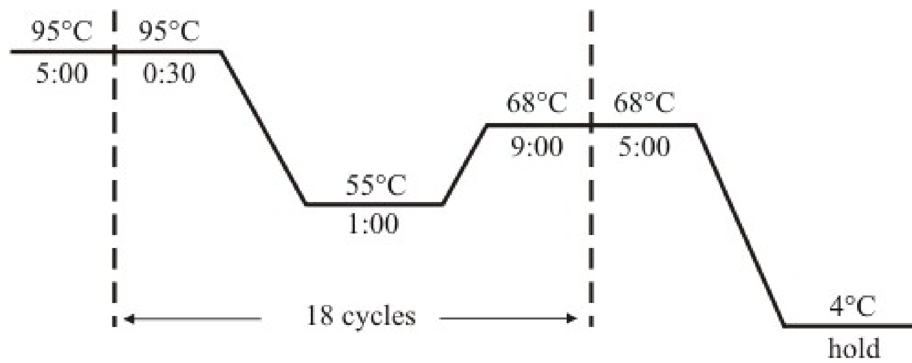
→ With this technique it is possible to change up to 3 base pairs (thus 1 amino acid; I never tried more, might work as well...). The procedure consists of an PCR step, during which the whole vector is amplified, followed by enzymatic digestion of the original vector and transformation of the unligated PCR product into chemically competent *E. coli* cells.



- design mutagenic oligonucleotide primers for the desired mutation:
  → $T_m \geq 78°C$ and 25- ~ 45 nt (Oligo Extinction Coefficient Calculator)
  → must contain desired mutation in approx. middle of the primer
  → primers must be complementary to each other
  → primers should end with one or more G or C
- vector must be *dam* methylated (isolated from e.g. DH5α or XL1blue, NOT JMxxx), vector should not be bigger than ~ 8000kb
- set up reaction as follows:

1	µL	plasmid	(~200-500 ng/µL)
1.25	µL	primer 1	(10 pmol/µL)
1.25	µL	primer 2	(10 pmol/µL)
2	µL	dNTP mix	(10 mM each)
5	µL	10x *Pfu* buffer	
38.5	µL	ddH_2O, mix well	
1	µL	*Pfu* DNA polymerase (Promega, M774A, 100u, 3 U/µL), mix well	
50	µL		

- use 0.2mL thin walled PCR tubes
- adjust elongation (and final extension) time to your vector length
  (rule of thumb: 1:00 for 1000bases, minimum 0:45! For big vectors add 1 min)



- add 1 µL DpnI (20 $^{U}/_{\mu L}$, NEB), 1h at 37°C (without shaking)
- transform 50 µL chemically competent cells (DH5α, XL1blue) with 1 µL / 5 µL mixture
- check for mutation by sequencing