mutagenesis-PCR (for base exchange)

- Primer must be individually chosen according to plasmid-backbone and DNA-fragment
- Insert desired base exchange
- Primer-T_m should be between 55 – 65 °C
- Primers should preferably have a GC-rich end (for a better adhesion to the plasmid)
- Dilute Primer 1:10 to avoid primer-dimerization
- Dilute template 1:5

mutagenesis-PCR approach	
	Volume in µl
Template (1:5)	15
dNTP´s each 100 nM	3.8
10x Pfu-buffer with $MgSO_4$	1
2 primer each 10 µM	0.8
Pfu-Polymerase	0.5 – 4
H_2O ad 50 µl	128.5

PCR-program			
	Temperature in °C	Duration	cycles
Denaturation	95	1 min	
Denaturation	95	0.5 min	
Annealing	Depends on primer	Depends on primer	
Elongation	Depends on primer	Depends on primer	
store	4	-------	

mutagenesis-PCR

- experiment date: _____; time: _____
- name of investigator: _____
- experiment date: _____; time: _____
- name of investigator: _____
- plasmid: name: _____ number: _____ production date: _____

Primer 1(forw): Primer 1 – sequence: Primer1 – T_M: Primer 1 – binding position:	Primer 2 (rev): Primer 2 – sequence: Primer 2 – T_M : Primer 2 – binding position:

mutanegesis-PCR approach	
	Volume in µl
Template (1:5)	
dNTP´s each 100 nM	
10x Pfu-buffer with $MgSO_4$	
2 primer each 10 µM	
Pfu-Polymerase	
H_2O ad 50 µl	

PCR-program			
	Temperature in °C	Duration	cycles
Denaturation			
Denaturation			
Annealing			
Elongation			
store		-------	