Colony-PCR

- Primer must be individually chosen according to plasmid-backbone and DNA-fragment
- Primer-T_m should be between 55 – 65 °C
- Primers should preferably end on GC (for a abetter adhesion to the plasmid)

- Put half of a clone from plate using a tip and transfer it in a 15 µl PCR-approach

Colony-PCR approach	
	Volume in µl
10x PCR-buffer	15
dNTP´s (each 100 nM)	3.8
DreamTaq-Polymerase (5 U/µl)	1
2 primer each 100 nM	0.8
H_2O	128.5
Σ-volume	150

PCR-program			
	Temperature in °C	Duration in sec	cycles
Denaturation	95	60	30
Denaturation	95	30	
Annealing	62-65	120	
Elongation	72	75	
End-elongation	72	300	
store	4	-------	

- Elongation time depends on the properties of the polymerase.

Colony-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:

Colony-PCR approach	
	Volume in µl
10x PCR-buffer	
dNTP´s (each 100 nM)	
DreamTaq-Polymerase (5 U/µl)	
2 primer each 100 nM	
H_2O	
Σ-volume	

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