Colony PCR

1. Pick a colony, make a replica on LB-plate with antibiotic. Transfer bacteria to 50µl of sterile water into 0.5ml tube. Vortex.
2. Boil for 5min. Vortex again.
3. Spin @ 11,000g for 1min to pellet down cell debris.
4. Use 10µl of supernatant for PCR (keep on ice).
5. Make a master mix for PCR:

MASTERMIX (multiply by number of analyzed colonies + a negative control: a colony from negative control plate):

H_2O	7.05
10xTAQ buffer	2.5
DMSO (5% final)	1.25
25mM $MgCl_2$	1.0
25mM dNTPs	0.2
10µM up primer	1.25
10µM dn primer	1.25
TAQ polymerase	0.5
	15 µl

Do NOT use high fidelity polymerase (such as Phusion)!

6. Add 15 µl of PCR master mix to each tube containing 10µl of colony supernatant.

Program: COLONY

- 95ºC – 2min
- 35 cycles:
    - 95ºC – 30sec
    - primer-specific anneal. TºC – 30sec
    - 72ºC – (1min per kb)
- 72ºC – 10min
- 4ºC.