

## Colony PCR

### Materials (per reaction):

- 5 µl water (Milli-Q)
- 0,5 µl primer Forward (10 pmol/ul = 10 mM)
- 0,5 µl primer Reverse (10 pmol/ul = 10 mM)
- 5 µl Phusion polymerase PCR Mastermix (NEB)
- PCR tube
- PCR machine

### Protocol:

Make sure that the PCR machine available.

Take the solutions from the freezer and thaw them on ice.

1. Pre heat a PCR machine to 98°C (to be used in step 5)
2. Gently mix the water and primers in a PCR tube after thawing
3. Keep solutions on ice
4. Pick a colony with sterile toothpick, dip the toothpick into the liquid in the PCR tube
5. Incubate 10 minutes at 98°C
6. Add the PCR Mastermix
7. Put the tube in the PCR machine and start the Colony PCR Program:

Colony PCR Program			
Step	Temperature	Time	Number of cycles
Initial denaturation	98°C	2 min	1
Denaturation	98°C	10 sec	30
Primer annealing	64°C *	10 sec	30
Elongation	72°C	30 sec**	30
Final elongation	72°C	5 min	1
Final	4°C	∞	1

\* Depends on your specific primers. Use an online tool to compute the annealing temperature.

\*\* Depends on the size (in kb) of your insert. Please refer to the manual of your polymerase.