

# Colony PCR

## Aim of the experiment

Polymerase chain reaction is an in vitro technique to exponentially amplify DNA of interest. Here, we exploit this technique to confirm the insertion of a specific DNA part into the genome of *E. Coli*. This is also referred as genotyping.

## Materials

- Template DNA
- Forward Primer (see list of sequences)
- Reverse Primer (see list of sequences)
- OneTaq Polymerase
- LB-Agar plate

## Procedure

1. Pick a colony.
2. Inoculate a numbered spot on a LB-Agar plate.
3. Inoculate a numbered spot on a LB-Agar plate with Antibiotics.
4. Mix the following reagents.

Concentration	Reagents
5µl	OneTaq buffer
0.25µM	Forward Primer
0.25µM	Reverse Primer
0.125µl	OneTaq Polymerase
bacteria from 1 colony	Template
Fill to 25µl	ddH2O

5. Transfer tube to a thermocycler with the following program.

Step	Temperature (°C)	Time (s)
Initial denaturing	95	300
25 -35 cycles	95	10
	Annealing Temperature	30
	72	30/kb
Final extension	72	300
Hold	4	forever