

# Colony PCR

## Introduction

The purpose of this protocol is to confirm correct insertion of DNA fragments after assemblies in the genome of *E.coli*.

## Two steps:

Preparation of template DNA from transformants  
Prepare and run PCR

## Information about the controls:

Negative control:

All the reagents in the master mix except the template (which is the plasmid with bioblocks).

If a band appears is an indicator of contamination.

Positive control:

The plasmid that was inserted in *E. coli* for amplification. The band should be smaller than the ones obtained from the colonies due to the lack of the added bioblocks.

## Materials

- 2x Taq DNA Polymerase Master Mix RED (from Ampliqon PCR Enzymes and Reagents)
- Forward Primer (10  $\mu$ M)
- Reverse Primer (10  $\mu$ M)
- MQ water
- Positive control (plasmid inserted in *E. coli* or colonies from transformation positive control)
- Template DNA (colonies to be tested)
- PCR tubes
- Eppendorf tubes
- Sterile toothpicks/ inoculation lops/pipette tips for transferring colonies
- LB media with appropriate antibiotics
- Transformants

## Procedure

### PCR mix and setup:

1. Pick a number of transformants, typically 3-10, from each plate of interest and mark them on the back of the plate.
2. Set up 2 PCR tubes for each colony and mark them accordingly.
3. Fill the first one (1) with 10  $\mu$ L MQ water.
4. Mix the PCR reaction in Eppendorf according to the number of colonies you wanna test including a positive and negative control:

Reactant	Per reaction (20 $\mu$ L) [ $\mu$ L]	Mix for 5 reactions [ $\mu$ L]
2x Taq DNA Polymerase Master Mix RED	10	50
10 $\mu$ M Forward Primer	1	5

10 $\mu$ M Reverse Primer	1	5
MQ water	8	40

5. Distribute the PCR mix in the rest of the PCR tubes (2) (plus a positive and negative control).
6. Transfer each colony to the PCR tube containing 10  $\mu$ L water using a sterile toothpick, inoculation loop or autoclaved pipette tip.
7. Transfer 1  $\mu$ L of the water from (1) to the PCR reaction (2) tube and mix.
8. Add 1  $\mu$ L of the template to the positive control and 1  $\mu$ L of MQ water for the negative control.
9. Run reaction in a thermocycler using the following settings.

1. Step	Temperature	Duration	Number of Cycles
Initial denaturation	95 °C	5 minutes	1 cycle
Amplification	95 °C	20 seconds	25-30 cycles (-0.5 °C per cycle)
	50-65°C	30 seconds	
	72 °C	around 1 min/kb*	
Final extension	72 °C	5 minutes	1 cycle
Hold	4 °C	-	1 cycle

10. Check the size of the PCR products in an appropriate agarose gel for positive results.
11. From the PCR tubes with colonies inoculated in the MQ water (1). From this you can save the colonies on the LB plate or inoculate in LB medium for an overnight culture.