

iGEM TU/e 2015
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2015.igem.org/Team:TU_Eindhoven

Colony PCR

Table of contents

Colony PCR	1	Colony picking	3
	2	PCR reaction	3
	3	Gel electrophoresis	4

1 Colony picking

Pick bacterial colonies (± 5) near the Bunsen flame with pipette tips. Load the colonies into Eppendorf tubes that are filled with 15 μL of sterile H_2O . Pipette up and down such that they are mixed well.

2 PCR reaction

The Master Mix should contain the following:

Component	Quantity/mass/final concentration	Volume (μL)
DNA (from bacteria)	Pipette tip in 15 μL H_2O	1
2x KAPA2G mix	1x	12.5
Primer FW	0.5 μM (stock: 10 μM)	1.25
Primer RV	0.5 μM (stock: 10 μM)	1.25
H_2O		9
Total		25

In order to simplify this step, prepare the master mix for all the colonies together wearing gloves.

Component	Quantity/mass/final concentration	Volume (μL)
DNA (from bacteria)	Pipette tip in 15 μL H_2O	1 for each separate PCR mixture
2x KAPA2G mix	1x	62.5
Primer FW	0.5 μM (stock: 10 μM)	6.25
Primer RV	0.5 μM (stock: 10 μM)	6.25
H_2O		49
Total		125

Run the following PCR program

Step	Temperature ($^{\circ}\text{C}$)	Time (sec)	Cycles
Denaturation	95	180 (3 min)	1
Denaturation	95	15	35
Annealing	58	15	
Extension	72	20 sec/kb)	
Final extension	72	600 (10 min)	1
Cooling	4	Hold	1

3 **Gel electrophoresis**

- After finishing the PCR program mix the samples with loading dye (1:6) and load 20 μL per well on the agarose gel
- Load appropriate ladder on the agarose gel
- Run the gel for ± 60 minutes on 100V

After colony PCR, perform plasmid amplification of the vectors that are correct.