Technische Universiteit
Eindhoven
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iGEM TU/e 2015
Biomedical Engineering

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Date
11 August 2015

Colony PCR

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Notebook

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

- 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 (°C)**	**Time (sec)**	**Cycles**
Denaturation	95	180 (3 min)	1
Denaturation Annealing Extension	95 58 72	15 15 20 sec/kb)	35
Final extension	72	600 (10 min)	1
Cooling	4	Hold	1

3 Gel electrophoresis

- After finishing the PCR program mix the samples with loading die (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.