Colony picking & colony PCR

PROTOCOLS IGEM TU EINDHOVEN

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iGEM TU Eindhoven 2019
Biomedische Technologie

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Content

1 Colony picking

Estimated bench time: 10 minutes per plate
Estimated total time: 10 minutes per plate
Purpose: Picking single colonies for further processing

It is essential to work sterile, thus disinfect your hands and work near a Bunsen Burner.

1.1 Materials

- Autoclaved Eppendorf tubes
- Autoclaved H_2O
- Bunsen Burner
- Pipettes & tips
- Plates with the bacterial colonies

1.2 Setup & protocol

- Fill out Eppendorf tubes with 15 µL autoclaved H_2O.
- Pick bacterial colonies (±5 per plate) near the Bunsen flame with a pipette tip.
- Place the colonies into correct labeled Eppendorf tubes.
- Pipette up and down such that they are mixed well.

2 Colony PCR

Estimated bench time: 30 minutes
Estimated total time: 10 minutes per plate
Purpose: Verifying if the colony contains the correct insert.

When performing a colony PCR, you are working with bacteria close to a Bunsen Burner. Make sure to disinfect your hands. When preparing the MasterMix, make sure to use gloves to protect the MasterMix from DNase activity.

2.1 Materials

- 2X KAPA2G mix
- Autoclaved H_2O
- Bucket with ice
- Pair of primers that correspond to correct binding sites on the vector
- PCR tubes
- Pipettes and tips
- Colonies from colony picking
- Thermal cycler

2.2 Setup & protocol

For one colony PCR reaction, the following mix should be made:

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	12.5
Primer FW	0.5 µM (stock: 10 µM)	1.25
Primer REV	0.5 µM (stock: 10 µM)	1.25
H_2O		9
Total		*25*

In order to simplify this step, prepare a MasterMix (2-3 reactions in excess). Keep the mix on ice. Do not add the bacterial DNA but take it into account while calculating the amount of required H_2O.

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	
Primer FW	0.5 µM (stock: 10 µM)	
Primer REV	0.5 µM (stock: 10 µM)	
H_2O		
Total		

Run the following PCR program:

Step	Temp (°C)	Time (s)	Cycles
Initial denaturation	95	180	1
Denaturation	95	15	35
Annealing	58	15	
Extension	72	20 s/kb	
Final extension	72	600	1
Cooling	4	Hold	1

3 Gel electrophoresis

Estimated bench time: 40 minutes
Estimated total time: 1.5 hours
Purpose: Agarose gel electrophoresis may be used to verify the purity of your PCR product. If the product is pure, a single bond will show up during the gel electrophoresis.

For more information, see our general Agarose Gel Electrophoresis protocol.