Technische Universiteit
Eindhoven
University of Technology

iGEM TU/e 2017
Biomedical Engineering

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Miniprep

Where innovation starts

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1 Miniprep

Estimated bench time: 1 hour
Estimated total time: 1 hour
Purpose: Plasmid extraction from the bacteria.

It is essential to work with gloves at all times to protect your plasmids from DNase activity.

1.1 Materials

- Autoclaved Eppendorf tubes
- Autoclaved H_2O
- Bacteria from which the plasmids are to be extracted (often in culture tubes)
- MiniSpin Centrifuge
- Pipettes and tips
- QIAprep Spin Miniprep Kit (QIAGEN)
- Tabletop Centrifuge

1.2 Setup & Protocol

- Centrifuge down the bacteria in the culture tubes for 10 minutes at 4,000 rpm (Tabletop Centrifuge). Make sure the tubes are well balanced.
- Discard the supernatant in the bacterial waste.
- Add 250 µl of buffer P1 to resuspend the pellets.
- Transfer the suspension to an Eppendorf tube.
- Add 250 µl of buffer P2 (make sure this buffer is not precipitated) to the mixture, which makes the solution turning blue.
- Invert the Eppendorf tube 4-6x to homogenize the solution. Do not vortex.
- After 2.5 minutes, within 5 minutes add 350 µl of buffer N3 and invert the solution 4-6x (do not vortex), allowing the solution to become clear and a ‘cloud’ of cell debris to form.
- Centrifuge the mixture for 10 minutes at 13,400 rpm (MiniSpin Centrifuge). A pellet of the cell debris will form.
- Pipette the supernatant off the pellets (carefully; do not transfer any pellet) and transfer it into a QIAprep spin column.
- Centrifuge the QIAprep column for 1 minute at 13,400 rpm. DNA will be bound to the column, discard the flow-through.
- Wash the column by loading 750 µl buffer PE and subsequent centrifugation for 1 minute at 13,400 rpm.
- Dry spin the column again for 1 minute at 13,400 rpm.
- Transfer the column from the collection tube to a new Eppendorf tube.
- Load 42 µl of autoclaved H_2O on the column (pipette drops in the middle of the membrane, do not touch the membrane). Incubate for 1 minute and centrifuge for 1 minute at 13,400 rpm.
- The resulting elution product will contain the DNA.