Technische Universiteit
Eindhoven
University of Technology

iGEM TU/e 2014
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
2014.igem.org/Team:TU_Eindhoven

Plasmid purification

MiniPrep

Technische Universiteit
Eindhoven
University of Technology

Table of contents

1 Plasmid purification

The plasmid DNA will be purified from the bacteria using the Qiagen QIAprep Spin Miniprep kit. Wear gloves to protect the DNA from DNAses! Also label well tubes and columns!

- Centrifuge down the bacteria in the culture tubes
  - Use one of the 'large' table-top centrifuges. Use the correct bucket inserts
  - Make sure all tubes are weight-balanced
  - Centrifuge for 10 minutes at 4000 rpm
- Discard the fluid atop of the pellet in the bacterial waste (autoclave boxes)
- Add 250 µL of buffer P1 to resuspend the pellets. Resuspend well
- Transfer the suspension to an eppendorf tube
- Add 250 µL of buffer P2 to the mixture, which makes the solution turning blue (cell lysis)
- Invert the eppendorf tube 4-6x to homogenize the solution. Do not vortex
- 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 (neutralization)
- Centrifuge the mixture for 10 minutes at 13,400 rpm in a 'small' benchtop centrifuge. A pellet of the cell debris will form
- Pipette the supernatant off the pellets (carefully) 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, so 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 fresh eppendorf tube
- DNA elution. Load 42 µL of water on the column (pipette drops in middle of 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 plasmid.
- Measure the concentration of the DNA using the NanoDrop Spectrophotometer ('Nucleic acid'). First measure blank with H_2O (and for initialization), and subsequently load 2 µL of DNA sample
- Store 10 µL of your plasmid in separate eppendorf tube for storage.