Technische Universiteit
Eindhoven
University of Technology

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

InterLab Study: Plasmid amplification

Technische Universiteit
Eindhoven
University of Technology

Table of contents

Plasmid amplification

1 Plasmid amplification

1.1 Subtract DNA from iGEM-kit

Estimated bench time: 10 minutes
Estimated total time: 20 minutes
Purpose: The dried DNA needs to be resuspended with water to be usable for transformation.

It is essential to work with gloves at all times to protect the DNA from DNase activity.

1.1.1 Materials

- Autoclaved H_2O (nuclease free water)
- Pipet and tips
- Well plates from iGEM Starter Kit

1.1.2 Setup & Protocol

- With a pipette tip, punch a hole through the foil cover into the corresponding well of the part that you want. Make sure you have properly oriented the plate. Do not remove the foil cover, as it could lead to cross contamination between the wells.
- Pipette 10 µl of dH_2O (distilled water) into the well. Pipette up and down a few times and let sit for 5 minutes to make sure the dried DNA is fully resuspended.
  It is recommended that you do not use TE to resuspend the dried DNA.

1.2 Transformation into NovaBlue

Estimated bench time: 30 minutes
Estimated total time: 90 minutes
Purpose: Placing the plasmid DNA into bacteria for amplification.

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

1.2.1 Materials

- Bucket with ice
- Bunsen Burner
- Incubator
- NovaBlue competent cells
- Heat/shaking-block
- Resuspended DNA (iGEM Starter Kit)
- SOC solution (Super optimal broth with catabolite repression)
- Water bath

1.2.2 Setup & Protocol

- Switch on the water bath and set temperature at 42 °C. Also turn on the heat/shaking-block and set up to 37 °C.
- Load a bucket with ice from the ice machine.
- Take the bacterial cells (NovaBlue) and SOC out of the -80 °C freezer. Transfer the cells directly to ice. Do not touch the bottom of the tube that contains the cells.
- Thaw the cells on ice for ~5 minutes.
- Add 1 ng of the resuspended DNA to 20 µl bacteria. Mix well. Make sure you work near the Bunsen burner flame.
- Leave the cells on ice for 5 minutes.
- Heat shock the cells for exactly 30 seconds at 42°C.
- Return the cells directly to ice for 2 minutes.
- Add 80 µl of SOC solution to the bacteria. Do not return on ice.
- Incubate for 1 hour at 37 °C and 300 rpm.
- Dry agar plate (supplemented with correct antibiotic) in the 37 °C incubator. Place the plate upside down and slightly opened.

1.3 Plating the cells

Estimated bench time: 30 minutes
Estimated total time: 18-20 hours
Purpose: Growing of the bacteria in which the plasmid is transformed.

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

1.3.1 Materials

- Agar plates
- Bunsen Burner
- Drigalski spatula
- Ethanol (70%)
- Incubator
- Pipette and tips

1.3.2 Setup & Protocol

- Take the dried agar plate out of the 37 °C incubator.
- Label the bottom of the plate with your initials, date, bacterial strain, plasmid type and gene name (mutant).
- Open an agar plate in close proximity of the Bunsen burner flame.
- Pipette 100 µl of the cells on the plate.
- Sterilize the Drigalski spatula by burning the alcohol on it (watch out that the burning alcohol does not ‘flow’ to your hands), shortly let it cool down.
- Spread the cells on the plate using the sterile spatula.
- Transfer the agar plate to the 37°C incubator. Place the plate upside down, closed.
- Let the cells grow on the plate overnight.

1.4 Colony picking

Estimated bench time: 15 minutes
Estimated total time: 18 to 20 hours
Purpose: To transfer the bacteria from the culture plates into larger culture tubes for further plasmid amplification.

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

1.4.1 Materials

- Antibiotic Stock
- Bunsen Burner
- Culture tubes
- Incubate orbital shaker 37ºC at 250 rpm
- LB medium
- Pipette and tips
- Pipetteboy and pipette

1.4.2 Setup & Protocol

- After taking the plates out of the 37 °C incubator place them upside down (i.e. the way they were in the incubator) on the bench top.
- Using a pipetteboy or similar instrument, pipette 8 ml of LB media containing the correct concentration of antibiotic into sterile cloning tubes (14 ml). Add 8 µl of antibiotic stock to each of the tubes. Make sure you work near the Bunsen burner flame.
- In one hand take a sterile pipette tip on the end of a pipette, with the other hand pick up the upside down plate containing the bacteria. Turn the plate over in your hand so that the bacteria are now facing upwards towards you and touch the tip of the pipette tip gently to a bacterial colony that is completely isolated from any other colony. Again work near the Bunsen burner flame.
- Now place the same tip with bacteria on it into one of the tubes containing LB media and move the tip around a bit to release some of the bacteria into the liquid. Some people simply eject the pipette tip into the media but if you do this you will need to recover it the next day.
- Culture the tubes overnight in an incubated orbital shaker at 37 °C at 250 rpm.

1.5 Glycerol Stock

Estimated bench time: 3 minutes per sample
Estimated total time: 3 minutes per sample
Purpose: To create a long-term stock of the bacteria with the correct plasmid.

It is essential to work sterile, thus disinfect your hands and work near a Bunsen Burner.
For working with liquid nitrogen it is important to wear cryo gloves.

1.5.1 Materials

- Bacterial Culture
- Bunsen burner

- Cryo tubes
- Glycerol (50%)
- Liquid nitrogen

1.5.2 Setup & Protocol

- Work near the Bunsen burner flame and mix 300 µl of 50% glycerol with 700 µl of the bacterial culture in cryo tubes.
- Snap freeze the samples in liquid nitrogen and transfer them to the -80 °C freezer.

1.6 DNA Extraction

Estimated bench time: 20 minutes
Estimated total time: 40 minutes
Purpose: To extract the plasmid DNA from the bacterial culture.

It is essential to work with gloves to protect the DNA from DNase activity.

1.6.1 Materials

- Cell culture
- Eppendorf tubes
- H_2O (nuclease free water)
- MiniSpin centrifuge
- Pipettes and tips
- QIAprep Spin Miniprep kit
- Table-top centrifuge

1.6.2 Setup & protocol

- Spin the cell culture in a table-top centrifuge 10 minutes at 4,000 rpm to pellet the cells, empty the supernatant (media) into a waste collection container.
- Resuspend pelleted bacterial cells in 250 µl Buffer P1 (kept at 4 °C) and transfer to an Eppendorf tube. No cell clumps should be visible after resuspension of the pellet.
- Add 250 µl Buffer P2 and gently invert the tube 4–6 times to mix. Do not vortex, as this will result in shearing of genomic DNA.
- After 2.5 and within 5 minutes, add 350 µl Buffer N3 and invert the tube immediately and gently 4–6 times. The solution should become cloudy.
- Centrifuge for 10 minutes at 13,400 rpm in a MiniSpin centrifuge. A white pellet will form.
- Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.
- Centrifuge for 1 minute at 13,400 rpm. DNA will be bound to the column, discard the flow-through.
- Wash QIAprep spin column by loading 750 µl Buffer PE and centrifuging for 1 minute at 13,400 rpm.
- Discard the flow-through, and centrifuge for 1 additional minute to remove residual wash buffer.
- Place the QIAprep column in a clean 1.5 ml Eppendorf tube. To elute DNA, load 42 µl water to the centre of each QIAprep spin column, let stand for 1 minute, and centrifuge for 1 minute at 13,400 rpm.

1.7 Nanodrop

Estimated bench time: startup time 5 minutes, time per sample 2 minutes
Estimated total time: startup time 5 minutes, time per sample 2 minutes
Purpose: To measure the concentration of the DNA.

It is essential to work with gloves at all times to protect the DNA from DNase activity.

1.7.1 Materials

- DNA samples
- Gloves
- H_2O (nuclease free water)
- NanoDrop spectrophotometer
- Pipettes and tips

1.7.2 Setup & Protocol

- Select the DNA measurement (Nucleic Acid) in the Nanodrop menu.
- Clear the surface of the Nanodrop with dH_2O.
- Preform a calibration and blank measurement by entering one drop of 2 µl dH_2O.
- Clean the surface again and place 2 µl per sample on the Nanodrop, and measure the amount.