iGEM TU/e 2015
Biomedical Engineering

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InterLab Study: Protein expression

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Protein expression

1 Protein expression

1.1 Transformation into BL21

Estimated bench time: 30 minutes
Estimated total time: 90 minutes
Purpose: Transformation of plasmid DNA into BL21 competent cells for protein expression.

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

1.1.1 Materials

- BL21(DE3) competent cells
- Bucket with ice
- Bunsen Burner
- Heat/shaking-block
- Incubator
- Pipettes and tips
- SOC solution (Super optimal broth with catabolite repression)
- Water bath

1.1.2 Setup & Protocol

- Prepare dilutions of the plasmid with a concentration of 1 ng/µl.
- 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 BL21(DE3 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 µl of the plasmid dilution to 20 µl BL21 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.
- Incubate for 30 minutes 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.

1.2 Plating the cells

Estimated bench time: 30 minutes
Estimated total time: 18-20 hours
Purpose: Letting the bacteria with the inserted plasmid grow.

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

1.2.1 Materials

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

1.2.2 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 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.

1.3 Colony picking

Estimated bench time: start up time 5 minutes and 3 minutes
Estimated total time: 18 to 20 hours
Purpose: Amplification of the bacteria with the inserted plasmid.

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

1.3.1 Materials

- Antibiotic Stock
- Bunsun Burner
- Culture tubes
- Incubate orbital shaker
- LB medium
- Pipette and tips
- Pipetteboy and pipette

1.3.2 Setup & Protocol

- After taking the plates out of the 37 ºC 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 mlL of LB media containing the correct concentration of antibiotic into sterile cloning tubes (14ml). Add 8µl of antibiotic stock to each of the tubes.
- 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 from the ligation. 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.
- 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 ºC at 250 rpm.
- Because the promotors used are constitutive no protein expression factor is needed to be added for protein expression.

1.4 Glycerol Stock

Estimated bench time: 5 minutes per sample
Estimated total time: 5 minutes per sample
Purpose: Preparing the bacteria for long term storage in the -80 °C freezer.

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.

1.4.1 Materials

- Autoclaved 50% glycerol
- Bacterial culture
- Bunsen Burner
- Cryo Tubes
- Liquid nitrogen
- Pipettes and tips

1.4.2 Setup & Protocol

- Fill Cryo Tubes with 300 µl 50% glycerol and 700 µl of the bacterial culture. Mix well.
- Snap freeze the samples in liquid nitrogen and transfer them to the -80°C freezer.