Technische Universiteit
Eindhoven
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iGEM TU/e 2015
Biomedical Engineering

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Double Transformation into BL21(DE3)

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1 Double Transformation into BL21(DE3)

Estimated bench time: 30 minutes
Estimated total time: 1.5 hour
Purpose: Amplification of the ligation product.

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

1.1 Materials

- Bucket with ice
- Bunsen Burner
- Eppendorf tubes with 20 ml competent BL21(DE3) cells
- Heat/shaking-block
- Incubator
- LB-agar plates supplemented with the correct antibiotic
- Pipettes and tips
- Plasmids to be transformed
- SOC solution (Super optimal broth with catabolite repression)
- Water bath

1.2 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.
- Prepare dilutions of the plasmids with a concentration of 20 ng/µl.
- Take the bacterial cells 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 plasmid 1 in each bacteria tube and add 1 µl of plasmid 2 to the bacteria tube. 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 (room temperature) to the bacteria. Do not return to ice.
- Incubate for 60 minutes at 37 °C and 300 rpm.
- Dry the LB-agar plates supplemented with the correct antibiotic in the incubator. Place the plates upside down (with the agar up) and slightly opened.

2 Plating of the cells on agar plates

Estimated bench time: 15 minutes
Estimated total time: 18 hour
Purpose: Amplification of the ligation product

For more information, see our general Plating protocol.