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Transformation into NovaBlue

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1 Transformation into NovaBlue

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 NovaBlue cells
- Heat/shaking-block
- Incubator
- LB-agar plates supplemented with the correct antibiotic
- Ligation mixture
- Pipettes and tips
- 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.
- Load a bucket with ice from the ice machine.
- 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 ligation mixture to 20 µl bacteria. Leave the bacteria on ice. Mix well. Make sure you work near the Bunsen burner flame.
- Incubate on ice for 5 min.
- 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 variable time at 37 °C and 300 rpm[1].
- Let excess water in the LB-agar plates with the correct antibiotics evaporate in the incubator (37 °C) . 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.

[1] Depends on what kind of antibiotic resistance you are working with, see manufacturer protocol for recommended incubation times.

For more information, see our general Plating protocol.