iGEM TU/e 2014
Biomedical Engineering

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Double transformation

In this protocol the double transformation of two plasmids in BL21 is described.

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1 Double transformation into E.coli BL21(DE3)

- Prepare dilutions of the plasmids with a concentration of 2 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 and SOC (Super optimal broth with catabolite repression) 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 each plasmid into 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 30 seconds (exactly!) at 42°C
- Return the cells directly to ice for 2 minutes
- Add 80 µL of SOC solution to the bacteria
- Incubate for 60 minutes at 37 °C and 300 rpm
- Dry agar plate, supplemented with chloramphenicol (25 µg/mL) and kanamycine (30 µg/mL) in the 37 °C incubator. Place plate upside down and slightly opened.

2 Plating the cells on agar plate

- Take the dried agar plate out of the 37 °C incubator
- Label the bottom of the plates
- Open an agar plate in close proximity of the Bunsen burner flame
- Pipette the cells (100 µL) on the plate
- Sterilize the Trigalski spatula by burning the alcohol on it, 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 .

3 Growth of small bacterial culture

- Fill 15 mL sterile culture tubes with 5 mL 2YT. Work near the Bunsen burner flame
- Add kanamycin (30 µg/mL) and chloramphenicol (25 µg/mL) to the cultures
- Pick a colony on the plate using a sterile pipette tip and pipette up and down a few times to ‘release’ bacteria (move pipette tip in solution)
- Grow the bacteria overnight at 37°C and 250 rpm

4 Prepare glycerol stock

- Prepare a glycerol stock of the bacteria that contain the plasmids
    - Mix 300 µL of 50% glycerol with 700 µL of the bacterial culture, in cryo tube
    - Snap freeze the samples in liquid nitrogen and transfer them to the -80°C freezer