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Biomedical Engineering

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Protein Expression

Where innovation starts

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1 Protein Expression

Estimated bench time: 60 minutes
Estimated total time: 17 hours
Purpose: Protein expression of the bacteria.

It is essential to work near the Bunsen burner at all times.

1.1 Materials

- 1.5 ml cuvettes
- 2YT-medium
- Aluminum foil
- Antibiotic stock(s)
- Arabinose (20%)
- Cell Density Meter (OD600)
- Fresh culture of bacteria containing the right plasmid(s).
- Incubator
- IPTG (1 M)
- Non-natural amino acid (10 mM)
- Pipettes and tips
- Sterile culture tubes

1.2 Setup & Protocol

- Prepare a culture tube containing 7 ml 2YT and 7 µl of both antibiotic stocks (ampicillin and chloramphenicol).
- Transfer 100 µl of the small culture to this new tube.
- Grow the bacteria in the incubator at 37 °C and 250 rpm.
- After 90 minutes: measure the OD600.
  OD measurement requires a blank measurement with 1 ml 2YT.
  Pipette 1 ml of the culture in the cuvette and measure the OD600.
- Put the culture back in the incubator (37 °C and 250 rpm). Regarding the fact that a cell division cycle takes around 20 minutes, calculate the amount of time the culture needs to obtain an OD600 of 0.6. (the OD600 doubles after ±20 minutes)
- After the additional time: measure the OD600 again. Pipette 1 ml of the culture in the cuvette and measure the OD600.
- The amount left in the culture should be 5 ml. When the OD600=0.6 wrap the culture tube in aluminum foil (only if you protein is sensitive to light) and add
  - 5.62 µl IPTG (1 M)[1]
  - 56.24 µl arabinose (20%)[1]
  - 562.42 µl non-natural amino acid (10 mM)[1]

  This makes the final concentration in the culture tube:
  - 1 mM IPTG
  - 0.2% arabinose
  - 1mM unnatural amino acid.
- Perform protein expression of ±15 hours at 18 °C and 250 rpm.

[1] It depends on your plasmid(s) what you need to add to initiate protein expression.