iGEM TU/e 2014
Biomedical Engineering

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Protein expression

General protocol for the incorporation of the unnatural amino acid

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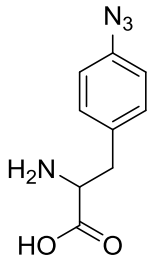
Protein expression

1 Growth of small bacterial culture

- Work near the Bunsen burner flame
- Fill a 15 mL sterile culture tube with 5 mL of 2YT
- Add 5 µL kanamycin (30 µg/mL) and 5 µL chloramphenicol (25 µg/mL) to the culture
- Pick cells from the glycerol stock using a sterile pipette tip and throw the pipette tip in the culture tube
- Grow the bacteria overnight at 37°C and 250 rpm

2 Stock solution p-Azido-L-Phenylalanine (pAzF)



Molecular Weight: 206.20
pAzF

Solution 10 mM pAzF

- 2yT medium
- DMSO: 5v%
- pAzF: 10 mM

→ shake at 4°C until all pAzF is soluted

3 Protein expression

- Transfer 100 µL of the small culture to new 7 mL 2YT culture
- Add 7 µL kanamycin (30 µg/mL) and 7 µL chloramphenicol (25 µg/mL) to the culture
- Grow the bacteria at 37°C and 250 rpm
- Grow until OD=0.6, OD measurement first requires blank measurement with 2YT
- Wrap culture tube in aluminium foil
- For a 5 mL culture: add 5.62 µL IPTG from the 1M stock, 56.24 µL of arabinose from the 20% stock and 562.43 µL of unnatural amino acid from the 10 mM stock to the culture wrapped in aluminum foil, in order to get end concentrations of 1 mM, 0.2% and 1 mM, respectively
- Perform protein expression for ~15 h at 25°C and 250 rpm

4 Preparation of cell dilution

- Spin down the cells for 15 min at 3,000 xg.
- Discard supernatant.
- Add 1 ml PBS and transfer to 1.5 mL Eppendorf microcentrifuge tube.
- Spin down the cells for 1 min at 13,400 rpm
- Discard supernatant.
- Add 1 ml PBS

- Fill 1 OD cuvette with 950 µL dH_2O and 50 µL PBS for blank OD measurement.
- Fill 1 OD cuvette with 950 µL dH_2O and 50 µL of culture sample (dilution of 20x)
- Measure OD, this has to be lower than 1, otherwise make a higher dilution.
- Calculate OD of culture sample. (If you made a dilution of 20X then the OD of the culture is 20X the OD of the dilution)
- Calculate amount of cells in culture http://www.genomics.agilent.com/biocalculators/calcODBacterial.jsp?_requestid=826255
- Your answer of the previous step is in bacteria/mL
  Calculate amount of PBS you have to add to obtain a final concentration of 10^7 cells/mL (in case of antibody titration) or 10^9 cells/mL (in case of reaction with DBCO). Use the following formula:

10^x cells/mL
This will give your dilution: $Dilution = \frac{\left[\frac{Bacteria}{mL}\right]}{10^x}$. **Amount of cells:** $Amount\ of\ cells\ (mL) = 1/Dilution$ because you will make 1 mL of 10^x cells **Amount of PBS:** $Amount\ of\ PBS\ (mL) = 1 - Amount\ of\ cells$ will make 1 mL of 10^x cells

- Store at 4 °C with aluminum foil.