Introductory tutorial to bacterial growth and kinetic assays in the Molecular Systems Biology Group

This tutorial serves as a practical introduction to the typical experimental work performed in the Molucalar Systems Biology lab.

1 Sterile technique

When working with microorganisms almost all work is done under sterile conditions to prevent contamination, and solutions prepared under non-sterile conditions are subsequently sterilised by autoclaving. Contaminating bacteria, yeast and fungi live on almost everything and float around in the laboratory air. When using sterile technique, the following guidelines apply:

1. Work in the laminar flow hood which provides positive pressure (make sure the sterilising UV light is turned off whilst working the hood, and on otherwise).
2. Always wear latex gloves OR regularly sterilise hands with a 70 % ethanol solution.
3. Flame bottlenecks with a bunsen burner whenever they are opened or used, and flame inoculating loops before each inoculation. Hockey-sticks should be kept in 70 % ethanol and flamed before use.
4. Only use autoclaved pipette tips.

2 Growing *Escherichia coli*

E. coli is typically grown in LB medium for bulk preparation of cell matter, however for reproducible physiological and/or kinetic studies a defined medium such as M9 minimal medium has to be used. The following recipes can be scaled to the desired volume.
To demonstrate the typical growth process of *E. coli* the following steps will be taken:

1. prepare either LB or M9 medium
2. inoculate a 5 ml culture from a freezer stock of *E. coli* and allow it to grow overnight (O/N)
3. use the O/N culture to inoculate a large culture and incubate on a shaker at 37 °C
4. monitor cell growth using a spectrophotometer

2.1 Lysogeny broth (LB) medium

This is a rich medium composed of tryptic digests of casein (peptides), yeast extract (vitamins, trace elements), and NaCl. As the exact composition of constituents in tryptone and yeast extracts is unknown and will vary per batch, LB medium is not recommended for physiological and kinetic studies, but rather for bulk growing of bacteria (e.g. for protein expression).
To prepare 1 l of LB medium, dissolve the following components in water (distilled water or preferably milli-Q) in a beaker with a magnetic spinner:

10 g	tryptone
5 g	yeast extract
10 g	NaCl

As a precaution it is possible at this stage to adjust the pH of the solution but is probably unnecessary. The solution can now be decanted into an Erlenmeyer flask with a cotton wool bung and foil covering the neck (note that for adequate aeration a 1 l culture should be grown in a 3 l Erlenmeyer flask) and autoclaved (usually at 121 °Cfor 15-20 min).

2.2 M9 minimal medium

M9 is a minimal medium with clearly defined components ideal for physiological and kinetic studies. Minimal media force the organism to produce the majority of the required metabolites, vitamins, co-factors endogenously and only provides the bare minimum of components exogenously in the medium. Unlike LB medium, M9 medium is buffered by the two phosphate components HPO_4^{2-} and $H_2PO_4^{1-}$. It is thus imperative that care is taken during preparation to include the correct forms of the phosphate salt!
To prepare 1 l of M9 medium, dissolve the following in 977 ml of water (milli-Q is preferred for reproducibility) in a beaker with a magnetic spinner:

12.8 g	$Na_2HPO_4.7H_2O$ **OR**
6.0 g	Na_2HPO_4
3.0 g	KH_2PO_4
0.5 g	NaCl
1.0 g	NH_4Cl

Also prepare a 20% w/v glucose solution in milli-Q water (typically glucose, sodium gluconate or glycerol are used as carbon sources); 500 ml is enough for 25 l of M9 medium. Glucose solutions may take a while to dissolve fully, but if left stirring with a magnetic stirrer the clumps will eventually disperse.
Transfer the glucose solution to a Schott bottle and the M9 salts to an Erlenmeyer flask with a cotton-wool bung and foil covering the neck of the flask (note that for adequate aeration a 1 l culture should be grown in a 3 l Erlenmeyer flask). Autoclave these solutions (usually at 121 °Cfor 15-20 min). The reason for autoclaving the salts and sugars separately is that when autoclaved together the sugars are typically caramelised, especially when phosphates are present.
While the glucose and salt solutions are being autoclaved, prepare the following two constituents. Once prepared, they should be filter sterilised using a 50 ml syringe and a 0.2 μm syringe filter into 50 ml Falcon tubes and stored at 4 °C (use a new filter for each solution):

- 50 ml 1 M $MgSO_4$
- 50 ml 0.1 M $CaCl_2$

To prepare 1 l of M9 medium add the following under sterile conditions to the 977 ml M9 salts autoclaved salts in the Erlenmeyer flask:

20 ml	20 % carbon source (0.2 % final)
2 ml	1 M $MgSO_4$
1 ml	0.1 M $CaCl_2$

As a precaution it is possible at this stage to adjust the pH of the solution which will be buffered around 7.2. This is generally unncecessary for growth of *E. coli*.

2.3 Batch growth

Typical aerobic bacterial batch cultures are grown by shaking in Erlenmeyer flasks.