Date;

**Large-scale protein expression**

This protocol uses a culture of 250-500ml. Unless you have a really good expression system, it’s not worth using smaller volumes of media. This volume of culture is handled conveniently by the centrifuges we have. The cell pellets produced are lysed in a volume which is easily passed through e.g. 1ml or 5ml affinity columns.

**Overnight culture inoculum**

Place e.g. 20ml LB in a 25ml “universal” or 50ml “Falcon” tube. Add the required antibiotic/s and vortex to mix. (Notes 1 and 2).

<table>
<thead>
<tr>
<th>volume added (ml)</th>
<th>stock conc. (µg/ml)</th>
<th>date prepared</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB</td>
<td>Ampicillin;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kanamycin;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Choramphenicol;</td>
<td></td>
</tr>
</tbody>
</table>

Transfer e.g. 5ml (1:100 overnight vol.:culture vol.) to each of three additional tubes.

Inoculate each 5ml LB medium (containing the appropriate antibiotic/s) with either a single colony or a scrape of cells from the surface of a -80°C glycerol freezer stock.

<table>
<thead>
<tr>
<th>Plasmid;</th>
<th>cell strain;</th>
<th>source of cells;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tape lids partly open and incubate overnight at 37°C, 200rpm.

Date;

**Larger volume cultures**

Add antibiotic/s to the required concentration/s to 500ml medium in 2l flasks (or 250ml in 1l flasks).

Minimal medium; ☐ LB; ☐ 2YT; ☐ autoinduction; ☐

<table>
<thead>
<tr>
<th>volume added (ml)</th>
<th>stock conc. (µg/ml)</th>
<th>date prepared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium;</td>
<td>Ampicillin;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kanamycin;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Choramphenicol;</td>
<td></td>
</tr>
</tbody>
</table>
Place flasks in the orbital incubator at 37°C, 200rpm for e.g. 30 minutes to warm.
Time at this point: 

Pour an overnight culture into each flask (5ml overnight/500ml fresh medium).
Continue incubation at 37°C, 200rpm.
Time at this point: 

Check cell growth by measuring the (apparent) absorbance at 600nm, against a 2TY medium blank. (Note 3). Protein expression is generally induced at A600 = 0.5 – 0.8, therefore starting checking cell density after e.g. 1.5 hours and 2 hours…

Spectrophotometer used; 

<table>
<thead>
<tr>
<th>A600 of each culture;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time; 1. 2. 3. 4.</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
<tr>
<td>3.</td>
</tr>
</tbody>
</table>

Reset incubator temperature, if required.
(Cool incubator more quickly with a flask of slushy ice.)

Take a sample of uninduced culture – e.g. 200μl. Pellet cells, 1 minute in a microfuge, remove supernatant. Resuspend cell pellet thoroughly in 50μl of 1x SDS-PAGE loading buffer.

Add IPTG to 0.4mM (0.1-1mM) from a 1M stock (in water, filter-sterilised).
stock concentration; [ ] mM; final concentration; [ ] mM;
volume added; [ ] μl.
Time at this point: 

Continue incubation, e.g. at 37°C, 200rpm for a further 3 hours, or as appropriate;
Temperature; [ ] °C. (see note 4).
Length of incubation; [ ] hrs

Pre-cool a centrifuge, ready for pelleting cells.

Place flasks on slushy ice for 10-30 minutes, swirling occasionally to cool.
Time at this point: 

Take a sample of induced culture – e.g. 100μl. Pellet cells, 1 minute in a microfuge, remove supernatant. Resuspend cell pellet thoroughly in 50μl of 1x SDS-PAGE loading buffer.

Transfer culture to centrifuge bottles and pellet cells at 4000g for 30 minutes at 5°C.
Time at this point: 

Decant supernatant and store cell pellets at -20°C, or cooler. (Note 5). (Dispose of the supernatant as GM waste, e.g. by adding Virkon to 1% (w/v)…).
(Cell pellets are resuspended in an appropriate buffer for cell lysis, determination of level of solubility (Note 6) and chromatography).
Notes

1. Especially when developing a new purification strategy, it is a good idea to prepare a number of cell pellets, each grown under the same conditions. Therefore, inoculate e.g. 20ml media + antibiotic/s with cells, vortex to mix and then aliquot 5ml to each of four 20ml Universals or 50ml Falcon tubes. Tape lids partly open and incubate overnight. As you try a new purification step, you can start with a new cell pellet, from the same batch, which should behave in the same manner as for previous trials.

2. Prepare the overnight cultures in tubes with a volume and width such that the medium can move and be well aerated; don’t use narrow, 15ml tubes. Using LB medium, rather than the richer 2YT, means the cells will grow more slowly and therefore spend less time at saturation and without selection having degraded the antibiotic/s.

3. You are measuring an *apparent* absorbance, due to light-scattering by the cells, rather than light absorption. As you are measuring light scattering, you may get different values from different spectrophotometers – this is due to different distances between the sample cuvette and the detector.

4. As a guideline for incubation temperatures;
   - 30°C – 3-5 hours
   - 25°C – 5-8 hours
   - 16°C – 8 hours to overnight.

5. Cells can be resuspended in e.g. 50ml sucrose 10% (w/v) and then re-pelleted to wash away media components and to allow cell pellets to be stored in smaller volume tubes than 500ml centrifuge bottles.

6. If the over-expressed protein is insoluble when over-expressed there are a number of changes to growth conditions which can be screened for effect on solubility;
   - Transfer flasks to 30°C, 20°C or 16°C after adding IPTG. Continue growth for e.g. 3 hours, 5 hours or overnight respectively.
   - Heat shock cells to 42°C for 15-20 minutes then place at 20°C and induce expression. Continue incubation for 4 -8 hours. (R.R. Burgess in Methods in Enzymology vol 463).
   - Add sucrose to the growth medium to 0.4M. (R.R. Burgess in Methods in Enzymology vol 463).
   - Add ethanol to the medium just before induction to 0.3, 1, 2 or 3% (v/v). Don’t add this before autoclaving as ethanol is volatile and will evaporate during autoclaving.
   - Add MgSO₄ to 0.1mM.

If all this fails to produce soluble protein, purification can be performed under denaturing conditions for some matrices and refolding can be attempted…