**Auto induced media preparation**

Based on F.W. Studier / Protein Expression and Purification 41 (2005) 207–234

**Procedure**

1. Pick one colony of freshly transformed bacteria into 10ml snap-cap tube containing 1-5ml auto-induced media
2. Grow over night (maximum 16h) in shaker at 37C and 30C.
3. Spin down 1.5ml aliquots in eppendorf tubes and aspirate media.
4. Freeze the dry pellets
5. Lyse the pellets in lysis buffer, sonicate, and run sup vs. pellet fractions for coomassie and western blot analysis (instructions for this section can be found here:

   - Multiple bacterial strains and media composition can be tested in parallel, using a 96 or 48 deep-well plates containing 0.5ml-1ml medium. (Please note that the shaker’s RPM should be changed according to the deep-well type)

**Composing the Auto-Induced media:**

**Materials**

**Formulation of ZYP-5052**: rich medium for auto-induction (this protocol is based on ZY media and it can be used with any other media, you can replace the ZY media with LB, TB or 2xYT for additional screening)

For all media, add 1M MgSO4 & 1000x metals mix before adding 20xNPS to avoid precipitate.

<table>
<thead>
<tr>
<th></th>
<th>1 litter total</th>
<th>final conc.</th>
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<tr>
<td>ZY (or 2xYT, LB etc.)</td>
<td>~928 ml</td>
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<tr>
<td>1M MgSO4</td>
<td>1 ml</td>
<td>1mM</td>
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<tr>
<td>50x5052</td>
<td>20 ml</td>
<td>1x</td>
</tr>
<tr>
<td>20xNPS</td>
<td>50 ml</td>
<td>1x</td>
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<td>antibiotic, <strong>as needed</strong></td>
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kanamycin (25 mg/ml)          4 ml          100ug/ml
chloramphenicol (25 mg/ml)    1 ml          25 ug/ml
ampicillin (50 mg/ml)         1 ml          50 ug/ml

Stock solution:
Use ddW for all solutions. Autoclave solutions for 15 min unless specified otherwise.

ZY
10 g bacto tryptone
5 g yeast extract
925 ml water

20xNPS: (NPS= 100mM PO₄, 25mM SO₄, 50mM NH₄, 100mM Na, 50mM K)
To make 100 ml:
90 ml water
6.6 (NH₄)₂SO₄ =0.5M
13.6 g KH₂PO₄=1M
14.2 g Na₂HPO₄=1M
add in sequence in beaker, stir until dissolve. pH of 20 fold dilution in water ~6.75.

50x5052: (5052= 0.5% glycerol, 0.05% glucose, 0.2% a-lactose)
To make 100 ml:
25 g glycerol (weigh in beaker)
73 ml water
2.5 g glucose
10 g α-lactose
add in sequence in beaker, stir until dissolve. can speed up by heating in microwave.

1M MgSO₄
24.65 g MgSO₄−7H₂O
water to make 100 ml