EXPRESSION OF RECOMBINANT PROTEIN IN BACTERIA

For use with all expression systems that require addition of IPTG.

Determine: optimum harvest time after induction
optimum growth temperature
optimum amount of IPTG needed for induction

Set up following variations:
- Room temperature uninduced control
- Room temperature with addition of 0.1 mM IPTG
- Room temperature with addition of 1 mM IPTG
- 37°C uninduced control
- 37°C with addition of 0.1 mM IPTG
- 37°C with addition of 1 mM IPTG

Day 1

1. Inoculate 2 x 5ml LB + antibiotic with 1 colony of transformed bacteria
   Grow 37°C o/n.

Day 2

2. Add to 6 falcon tubes:
   8.5 ml fresh LB + antibiotic
   1.5 ml overnight culture

   Grow at room temperature or 37°C until OD_{600} = > 0.6

3. Record absorbance and take 1 ml samples from each tube (T_0). Spin 13K 1' in a microfuge and discard supernate. Snap freeze the pellet in liquid N_2 and store –80°C.

4. Induce cells by addition of IPTG:
   1mM IPTG = 12µl of a 20% stock to each culture
   0.1mM IPTG = 12µl of a 2% stock to each culture

5. Take 1 ml samples from each tube at four different time points (T_{1-4}). Check OD_{600} and then treat as described for T_0. The absorbance readings will indicate if the cells are still dividing, stationary or dying. The optimum situation is one in which no cell growth or division is occurring so that the energy is going into production of the recombinant protein.

Day 3

6. Run acrylamide gel of time course to determine optimum conditions for
   protein production. Add 100 μl **1 x protein loading dye** to each pellet,
   boil 5' and load 10μl.

1 X protein loading dye (20ml) (make fresh)

1% SDS	2 ml 10%
100 mM β-mercaptoethanol	138 μl
0.1% bromophenol blue	2 ml 1%
100 mM Tris pH 8.0	1 ml 2 M
10% Ficoll	2 g