Recombinant expression and SDS-PAGE of Proteins

1. Obtain a bacterial culture containing a Protein X (Lec-1) expression vector from your TA. There are two types, A and B – one is grown in the absence of arabinose, one has been arabinose induced – it is your job to work out which is which.

2. Take 500µl of bacterial culture and pipette it into a separate 1.5ml tube, centrifuge the tubes for 2 minutes to pellet the bacterial cells. Label your tubes with your name and write down which culture number you have in your notebook and on the tube.

3. Carefully remove the growth media with a pipette leaving the bacterial pellet on the bottom of the tube.

4. Add 100 µl of SDS-PAGE sample loading buffer to each bacterial pellet and mix by gently pipetting the sample buffer in and out of the pipette tip. Note: do this **SLOWLY** so as not to cause the sample buffer to foam.

5. Incubate the bacteria/sample buffer mixtures at 100 °C for 5 minutes in the heating block.

6. Load 25 µl of each sample onto the SDS-PAGE gel provided by your instructor.

7. The gel will be run for 30 minutes at 200 volts.

8. When the gel is finished, carefully separate the glass plates using a spatula. The gel should stick to one of the plates.

9. Place the polyacrylamide gel in a plastic or glass container. Cover the gel with 3 to 5 gel volumes isopropanol fixing solution and shake gently at room temperature for 10 min.

10. Pour out fixing solution. Cover the gel with rapid Coomassie blue staining solution and shake gently until desired intensity is reached. Bands should become visible in the staining solution within 5 to 30 min.

11. Pour out staining solution. Cover the gel with 10% acetic acid to destain, shaking gently until a clear background is obtained.

12.The TA will take the gel pictures.

SDS-PAGE sample buffer (2X)
0.125 M Tris-CL pH 6.8
4% SDS
20% glycerol
2% 2-mercaptoethanol

0.01% bromphenol blue

Acrylamide Solutions for Two Laemmli Gels	15%	4%
30% Acrylamide	5 ml	0.65 ml
4X Tris-HCl, pH 8.8	2.5 ml	
4X Tris-HCl pH 6.8		1.25ml
ddH2O	2.5 ml	3.0 ml
10% Ammonium Persulfate	33 µl	50 µl
TEMED	7 µl	7 µl

SDS electrophoresis buffer, 5×
15.1 g Tris base (0.125 M final)
72.0 g glycine (0.96 M final)
5.0 g SDS [0.5% final]
H_2O to 1000 ml

Isopropanol fixing solution
25% (v/v) isopropanol
10% (v/v) acetic acid
65% H_2O

Rapid Coomassie blue staining solution
10% (v/v) acetic acid
0.001% (w/v) Coomassie brilliant blue R-250 (dissolved in MeOH or EtOH)
90% H_2O