PROTOCOL TO GENERATE WHOLE CELL EXTRACTS FOR SDS-PAGE

- Resuspend pellet from one well of a six well plate into 80-100 ul of lysis buffer * (200 ul 1M Tris-HCl pH 7.5, 1 ml 10% SDS, 2 ml 50% glycerol in 10 ml) containing 2X the concentration of protease inhibitors (calbiochem).

- Boil samples 5 minutes. Put on ice.

- Shear DNA with a 1ml syringe with a 23 5/8, 25 5/8 or 26 5/8 needle.

- Centrifuge 10 minutes at 14 000 rpm. Keep supernatant.

- Determine protein concentration and load on gel.

* For T-150, use larger volumes like 300-500 ul lysis buffer depending on the size of the pellet.

Eric Campeau V. 1.0 (original protocol obtained from Dr. Janice Pluth, Lawrence Berkeley National Laboratory).