Analysis of total E. coli protein by SDS PAGE

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1. In microfuge tubes, spin down 0.1 ml of uninduced cells grown to near saturation or 0.15 ml of IPTG induced cells. Remove YT (or LB) media with a pipetman or drawn out pasteur pipette and freeze cell pellets for later analysis.

2. Add 120 microliters 1X SDS PAGE loading buffer to cell pellet and vortex until cells are resuspended.

3. Boil cells 5 min in H2O bath or heat 5 min in 95 degree heating block.

4. Spin tubes in microfuge for 5 minutes.

5. Load 6-10 microliters to Invitrogen NuPAGE gels (or other suitable SDS PAGE gel) and stain with Coomassie blue.

Note: Boiled cells can be viscous due to the cellular DNA. If very viscous (hard to load on gel), try to pipet the SDS PAGE loading buffer from a region of the tube with a low viscosity.
1 X SDS PAGE loading buffer

NuPAGE loading buffer (120 microliters):
30 microliters 4X NuPAGE loading buffer (heat in warm H2O for a few minutes)
60 microliters 0.1 M DTT (16 mg/ml)
30 microliters H2O