PROTEIN BIO-DOT ('SLOT BLOT')

Adapted from existing protocols by Vinh Pham.
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MATERIALS:

Cracking buffer stock solution

Urea	**48g**	
SDS	**5g**	
1M Tris-HCl, pH 6.8		**4ml**
0.5M EDTA	**20ul**	
Milli-Q H_20	**100ml**	

Complete Protease Inhibitor soln. (dissolve 1 tablet in 2ml H_20, store at –20 deg. C)

PMSF (1.7mg/ml)
Glass beads (424um-600um)
2-Mercaptoethanol
Nitrocellulose membrane
Whatman paper
Bio-Dot SF Filter Paper 60
Non-fat dry milk
Tween-20
1X TBS

PROCEDURE:

Part I - Sample Preparation

1. Grow cells to desired concentration (OD600 of 1.6-2.0 for *E. coli* cultures).
2. Calculate OD600 units (=OD600 X volume). *M. xanthus* cultures at Klett 90, 100, 120 have OD600 readings of 0.560, 0.700, and 0.900, respectively.
3. Pellet at 14Krpm for 1-2'.
4. Freeze with liquid N_2.
5. Store at –80 deg. C.
6. Make fresh cracking buffer (CB).

Cracking buffer stock soln	3ml	1ml	500ul
2-ME (*optional – use only for inclusion bodies)*	30ul	10ul	5ul
Protease Inhibitor cocktail	210ul	70ul	35ul

7. Warm CB to 60 deg. C.
8. Add 150ul 1.7mg/ml PMSF (*optional*) or dH_2O to CB (makes CBP).
9. Add at least 100ul CBP per 7.5 OD600 units of cell pellet (V=100ul x OD600 units/7.5). Resuspend by vigorous pipetting and/or incubation at 60 deg. C for 2'.
10. Add ~80ul glass beads per 7.5 OD600 units of cell pellet. (Use weighing paper to add desired quantity of beads to a separate tube, then transfer contents to cell lysate.)
11. 70 deg. C for 10'.
12. Vortex 1'.
13. Pellet 5' at max speed, 4 deg. C.
14. Transfer s/n to new tubes and freeze at –20 deg. C. Avoid transferring glass beads and gunk embedded in glass beads.
15. Boil remaining pellet at 100 deg. C for 5'.
16. Vortex 1'.
17. Pellet 5' at max speed, 4 deg. C.
18. Combine s/n with s/n from step 14.
19. Sonicate at 70%-90%, 7X-10x, for 1-2 minutes to shear the DNA.
20. Measure [protein] by Lowry method using Bio-Rad *DC* Protein Assay.

Part II – Slot Blotting

1. Cut nitrocellulose membrane and 3 pieces of Whatman paper (use more if bleeding of samples is a problem) to size of Bio-Dot SF filter paper 60.
2. Soak membrane and filter papers in TBS.
3. Touch membrane on dry filter paper to wick off excess liquid.
4. Place into Bio-Dot SF apparatus:

TOP — Sample template with screws
Nitrocellulose membrane

Filter paper 60
Whatman paper
Whatman paper
Whatman paper
Gasket
Gasket support plate
BOTTOM Vacuum manifold

5. Press out bubbles with glass pipet.
6. Turn on vacuum and tighten screws in diagonal pattern.
7. Apply atmosphere pressure.
8. Rehydrate membrane with 100ul TBS per well.
9. Apply vacuum/atmosphere pressure.
10. Boil samples 10'.
11. Cool on ice ~2'.
12. Apply samples (use at least 200ul, but no more than 500ul). Maintain vacuum/ atmosphere pressure to ensure even distribution of sample and to avoid bleeding.
13. Wash each well 2X with 100ul TBS by vacuum/atmosphere pressure.
14. Remove membrane (remove sample template while vacuum/atmosphere pressure is applied, turn off vacuum, remove membrane).

Part III – Detection

1. Block membrane in 5% non-fat dry milk/0.1% Tween-20 in TBS for 1 hour at RT.
2. Wash 2X with TBS-Tween-20 (TBST) for a few seconds, 1X for 15', 2X for 5'.
3. Dilute primary antibody in TBST (e.g., 1:1000 is 10ul in 10ml TBST).
4. Add washed membrane to primary antibody soln, incubate at RT for at least 1 hour.
5. Wash as in step 2.
6. Dilute secondary antibody in TBST.
7. Add washed membrane to secondary antibody soln, incubate at RT for at least 1 hour.
8. Wash as in step 2.
9. Wash 2X in TBS for 5' to remove Tween-20.
10. Rinse 2X briefly in distilled water (this helps to remove all residual Tween-20).
11. Warm ECL+ reagents to RT (mix cold A and B solns in ratio of 40 to 1 in a 15ml tube and warm to RT).
12. Place membrane on saranwrap, protein side up.
13. Add detection mixture to top of membrane, incubate at RT for 5'.
14. Wick off excess detection fluid and tape to old piece of x-ray film to hold in place.
15. Scan on STORM using Blue Fluorescence/Chemiluminescence mode.