Two-dimensional electrophoresis of 30S protein (Xinying Shi)

Reference:

1. Geyl D et al, 1981. An improved method for two dimensional gel-electrophoresis : analysis of mutationally altered ribosomal proteins of Escherichia coli. Mol Gen Genet 181:309-312
2. Siegmann & Thomas, 1987. Separation of multiple phosphorylated forms of 40s ribosomal protein S6 by two dimensional polyacrylamide gel electrophoresis. Methods Enzymol 146:362-369

Stock solutions

- 30% Acrylamide/bisacrylamide (ratio 29:1) (Bio-rad)=29%acrylamide, 1%bisacrylamide
- 10% ammonium sulfate (APS)
- Loading buffer: 8M urea,1% BME,10mM Bis-tris-acetate,pH4.2
- 100mM Bis-Tris-acetate (pH5.5): use for 1D gel
- 50mM Bis-Tris-acetate (pH3.8): use for 1D running buffer (+) 1:5 dilute when use
- 50mM Bis-Tris-acetate (pH6.0): use for 1D running buffer (-) 1:5 dilute when use
- 2M KOH-acetate (pH4.6) use for 2D gel
- 744mM glycine-acetate (pH4.0) use for 2D running buffer 1:4 dilute when use
- Saturated water solution of basic fuchsin (10mg in 1ml water—1%)
- 0.25% Coomassie Blue in 45% Methal,10% acetic acid and 45% water

Protein extraction

(all the procedures were done at 4C)

- Take 100 pmol 30S subunit and make it to 23 µl by adding water.
- Add 0.1 volume of 1M Mg acetate (final will be 0.1M Mg, so add 10 µl) and 2 volume glacial acetic acid (so add 67 µl). Final volume is 100 µl.
- Mix for 45min at 4C in the Eppendorf mixer.
- Centrifuge at 16,000g for 10min
- Transfer the supernatant to a new tube.
- Add 4 volumes of acetone and precipitate overnight at -20 C
- Centrifuge at 10,000g for 10min
- Remove the residual acetone by placing for 30min in a dessicator (RT).
- Dissolved in 10 ul loading buffer (8M urea,1% BME,10mM Bis-tris-acetate,pH4.2)
- 37 C for 30min (Don’t boil samples containing urea!)
- Add 1ul water saturated basic fuchsin dye
- Load 100 pmol/lane to a well

1st dimension:

Gel: 8M urea,
4% acrylamide,
0.14% bisacrylamide
40mM Bis-Tris-acetate,pH 5.5
0.03% APS+0.1% TEMED, polymerize for 30min

Loading buffer: 8M urea,1% BME,10mM Bis-tris-acetate,pH4.2
Upper buffer(+): 10mM Bis-Tris-acetate (pH3.8) inner chamber,
Lower buffer(-): 10mM Bis-Tris-acetate (pH6.0) outer chamber
Electrophoresis:
Invert the electrodes, sample migrate from anode(+) to cathode(-)

Wash plates before running the gel. Perform with a direct current of 8mA/gel for 2h 45min until dye reach to the edge of the plates. Run gel at room temperature.

Immediately after the completion of the first dimension, the gel slabs are cut and placed lengthwise at the bottom of the glass plates.

2nd dimension:

Gel: 6M urea
18% acrylamide
0.62% bisacrylamide
48mM KOH-acetate at pH4.6
0.05% APS and 0.1% TEMED,
pour over the first dimension gel slab to the top of the short plate.
Gel running buffer: 186 mM glycine-acetate, pH4.0
Electrophoresis
Don't invert electrode! Sample migrate from anode(+) to cathode(-).

Electrophoresis is performed with a direct current of 8mA/gel for 1h 45min until dye reach to the edge of the plates. Run gel at room temperature.

The second dimension gel is stained overnight in 0.25% coomassie brilliant blue 10min with stain buffer.
Scan wet gel by placing gel between two transparency films. Scan at 600 DPI resolution on EPSON scanner with Photoshop. Save as PSD file.

	Stock solution	*Volume*	*Running buffer*
8M urea(FW=60)	-	4.8g	+ (upper) 10mM
4.0% acrylamide	29%acrylmide, 1%bisacrylamide	1.38ml	Bis-Tris-acetate
0.14%bisacrylamide	-		(pH3.8)
40mMBis-Tris-acetate,pH 5.5	100m MBis-Tris-acetate,pH5.5	4ml	
0.15% APS	10%	0.15ml	- 10mM
0.15% TEMED	100%	0.015ml	Bis-Tris-acetate
water		0.62=10ml	(pH6.0)
6M urea	-	3.6g	
18% acrylamide	29%, 1%	6.2ml	186 mM
0.62% bisacrylamide	-		glycine-acetate
48mM KOH-acetate, pH4.6	2M KOH-acetate (pH4.6)	0.24ml	pH4.0
0.1% APS	10%	0.1ml	
0.1% TEMED	100%	0.01ml	
water		0.45=10ml	

Stock suolutions	**weight**	**Final volume**
100mM Bis-Tris-acetate (pH5.5)	4.18g	200ml
50mM Bis-Tris-acetate (pH3.8)	10.45g	1000ml
50mM Bis-Tris-acetate (pH6.0)	10.45g	1000ml
2M KOH-acetate (pH4.6)	5.6g	50ml
744mM glycine-acetate (pH4.0)	55.8	1000ml
50mM bis-tris-acetate,pH4.2	0.52	50ml
loading buffer		
8M urea		480mg
1% BME,		10ul
10mM Bis-tris-acetate,pH4.2	50mM	200ul
water		400 =1ml
		Add 1ul BME to100ul

Bis-tris FW=209
Glycine FW=75
KOH FW=56
Urea FW=60