Mobility Shift Assay

2/21/94 Takeshi

1. Prepare the gel.
   1) Prepare the following mixture.

	for	1 gel	2 gel
10 x Tris Glycine		4.0 (ml)	8.0 (ml)
30 % Acrylamide		5.33	10.66
2 % Bisacrylamide		1.0	2.0
50 % Glycerol		2.0	4.0
qH_2O		27.67	55.34
	total	40 ml	80 ml

   2) Filter through 0.2 um filter and vacuum for 15 min. Add 30 % ammonium persulfate 100 ul (for 2 gels, 250 ul or more) and TEMED 34 ul (for 2 gels 68 ul).

2. In 1 x Tris Glycine buffer, pre run gels at 100 V for one to two hours.
3. Prepare the binding reaction.
   1) Prepare DNA binding buffer.
      Add 1 ul of 1 M DTT and 4 ul of 0.25 M PMSF to 1 ml of stock.
   2) Prepare DNA binding reactions.

BSA (4.5ug/ul)	1 ul	
Poly dIdC (1 ug/ul)	2 ul	
Nuclear Extract	x ul	
DNA binding buffer	17-x-y(-z) ul	
Competitor (if necessary)	(z ul)	
^{32}P probe	y ul / 60,000 cpm	(in general, 20,000-100,000 cpm)

   3) Incubate at RT for 15 min.
4. Load samples onto the gels and run gels at 10 mA/gel (which allows gels to run for 1.5-2 hours) until bromophenol blue dye front reaches 2-3 cm (for 30 mer probe) from the bottom of the gel.

Solutions

10 x Tris Glycine

Tris base	30.28 g	
Glycine	142.7 g	
EDTA	3.92 g	
qH_2O	up to 1 l	Adjust pH with HCl to 8.5

DNA binding buffer

	final	stock	for 100 ml	
Hepes (pH7.9)	12 mM	1 M	1.2 ml	
Tris/HCl (pH7.9)	4 mM	1 M	0.4 ml	
KCl	60 mM	1 M	6.0 ml	
EDTA (ph 8.0)	1 mM	0.5 M	0.2 ml	
Glycerol	12 %	50 %	24 ml	
qH_2O			68.2 ml	
PMSF	1 mM	0.25 M		PMSF and DTT should be
DTT	1 mM	1 M		added just before use.

Ref: Chodosh, L.A., "Current Protocols in Molecular Biology" unit 12.2

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3. Prepare the binding reaction.
   1) Prepare DNA binding buffer.(Add 1 ul of 1 M DTT and 4 ul of 0.25 M PMSF to 1 ml of stock.)

	Probe (x)	Nuclear Extract (y) (+ Dialysis Buffer)	Buffer (17-x-y)	Comment
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				

	Probe (x)	Nuclear Extract (y) (+ Dialysis Buffer)	Buffer (17-x-y)	Comment
13.				
14.				
15.				
16.				
17.				
18.				
19.				
20.				
21.				
22.				
23.				
24.				

2) Prepare DNA binding reactions.

BSA	1ul
Poly dIdC	2 ul
Nuclear Extract	x ul
^{32}P probe	y ul
DNA binding buffer	17-x-y ul

3) Incubate at RT for 15 min.
4) Load onto gels.

4. Run gels at ( ) mA/gel until bromophenol blue dye front reaches 2-3 cm from the bottom of the gel.
   ( )