Gel Mobility Shift Assay Conditions -Mg/EDTA in Gel and Buffer

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Protein Dilution Buffer	**5ml**
20 mM Tris pH7.9	100 microliters 1M Tris 7.9
150 mM KCl	0.75 ml 1 M KCl
1 mM DTT	50 microliters 0.1 M DTT
10% glycerol	1 ml 50% glycerol
50 micrograms/ml BSA	2.5 microliters 100 mg/ml BSA
3.1 ml H2O	

Optional: add Brij 58 to 0.1%.

Store dilution buffer at -70 degrees.

Proteins are diluted in dilution buffer and quick frozen on dry ice. Thaw proteins on ice. Proteins are typically stable to multiple repeated freeze thaw.

5x binding buffer	**1 ml**
20% glycerol	400 microliters 50% glycerol
100 mM Tris-HCl pH8	100 microliters 1 M Tris pH8
300 mM KCl	300 microliters 1 M KCl
25 mM MgCl2	25 microliters 1 M MgCl2
500 micrograms/ml BSA	5 microliters 100 mg/ml BSA
	170 microliters H2O

optional: add 25 microliters saturated bromophenyl blue [BioRad] (~0.1% in H2O) per ml of 5X buffer (this may inhibit the binding of some proteins)

Store Buffer at -20 deg.

Gel shift reactions are performed as follows:

- 20 microliter binding reaction:
- 4 microliters 5X binding buffer
- 0.2 microliters 0.1 M DTT
- 2000-5000 cpm labeled DNA
- 0.125 micrograms p[dG-dC]
- H2O to 20 microliters final volume

Add proteins to reaction last. Incubate protein and DNA at room temperature for ~30-40 min and load to native gels which are run in the cold room at 4 degrees. Gels are not pre cooled but are set in cold room 5-10 minutes before loading and pre run at 160 V. The wells of the gels are rinsed out several times before prerunning and again before loading. Samples are applied to the gel while the gel is running. For best results, use a fine tip pipetman tip to load the gels. We run gels at 160V (12 cm long) for ~45 min.

For a typical gel shift reaction (20 microliter reaction), I use 1-2 ng TBPc (the conserved region of yeast TBP from the Sigler lab) and and 5-10 ng of wild-type or truncated yeast TFIIB. The amount of proteins will have to be titrated for your specific conditions.

Gels:

10.5 ml (20%/0.33%) acrylamide/bis acrylamide
3.5 ml 10X TGOE buffer
1.75 ml 50% glycerol
35 microliters 0.5 M DTT
20.9 ml H20
0.3 ml 10% ammonium persulfate
30 microliters TEMED

10X TG0E	**500 ml**
0.25 M Tris	15.1 g Tris
1.9 M glycine	71.3 g glycine

pH 8.3 with acetic acid at room temp.
Adjust volume to 500 ml.

Running buffer is 1X TGOE buffer

[Note that there is no Mg or EDTA in both the gel and in the running buffer.]