GEL MOBILITY SHIFT ASSAY

Material/reagents:

- 2X binding buffer:
  20 mM Tris pH 7.5
  100 mM NaCl
  2 mM EDTA
  10% glycerol
- poly(dI-dC) (5 μg/μl in TE)
- BSA (10 mg/ml)
- ^{32}P-labeled probe
- Buffer C:
  20 mM HEPES pH 7.9
  25% glycerol
  420 mM NaCl
  1.5 mM $MgCl_2$
  0.5 mM DTT
  0.5 mM PMSF
  0.2 mM EDTA
- 4% native polyacrylamide gel (50 ml):
  5 ml 40% acrylamide (30:1)
  2.5 ml 5X TBE
  41.95 ml H_20
  0.5 ml 10% APS
  50 μl TEMED
- 0.25X TBE running buffer
- Loading dye:
  0.2% bromophenol blue
  0.2% xylene cyanol
  50% glycerol

Procedure:

1. Prepare reaction mix (20 μl/sample):

dH_20	6.3 μl
2x binding buffer	12.5 μl
BSA (10 mg/ml)	1.0 μl
poly(dI-dC) (5 μg/μl)	0.1μl
probe (1 ng/μl)	0.1μl

2. Add buffer C to the reaction mix if your volume of whole cell extract will be less than 5 μl. The sum of whole cell extract and buffer C should be 5 μl.

3. Add 10–15 μg of whole cell extract (≤ 5 μl) to the reaction mix and resuspend. The total volume should be 25μl.

4. Incubate at room temperature for 20 min.

5. Add 2.5 μl of loading dye.

6. Load sample onto a 4% polyacrylamide gel.

7. Run the gel at room temperature for ~2.5 hours at 120-130 V in 0.25X TBE. (Run until the bromophenol blue is ~1 inch from the bottom of the gel. Free probe will co-migrate with the blue dye.)

8. Dry the gel at 80°C and expose to film or PhosphoImager.

Comments:
This protocol works well for HSF DNA-binding activity experiments. An alternative protocol by Gen Matsumoto uses a 19:1 40% polyacrylamide and no BSA, but is otherwise the same.

References:
1. Mosser et al., 1988, Mol. Cell. Biol. 8:4736-4744.
2. Fried and Crothers, 1981, Nucleic Acids Res. 9:6505-6525
3. Garner and Revzin, 1981, Nucleic Acids Res. 9:3047-3060
4. Singh et al., 1986, Nature 319:154-158

Submitted by:
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