**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)
- 32P-labeled probe
- Buffer C:
  - 20 mM HEPES pH 7.9
  - 25% glycerol
  - 420 mM NaCl
  - 1.5 mM MgCl<sub>2</sub>
  - 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<sub>2</sub>O
  - 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<sub>2</sub>O 6.3 μl
   - 2x binding buffer 12.5 μl
   - BSA (10 mg/ml) 1.0 μl
   - poly(dI-dC) (5 μg/μl) 0.1 μl
   - probe (1 ng/μl) 0.1 μl

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 25 μl.

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 PhosphorImager.

**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:**

**Submitted by:**
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