ELECTROPHORETIC MOBILITY SHIFT ASSAY (EMSA)
Adapted from BV's Cookbook, TCH 1/27/02

Useful for analyzing whether a given protein (usually recombinant), or proteins, can specifically bind to a radiolabeled DNA probe. Because you're reading this, we'll assume you already have (a) a reasonably pure (>85% pure by Coomassie staining) preparation of your protein and (b) have at least identified a consensus sequence to which your protein is likely to bind.

Making/labeling probe:

The simplest way to get started is to order two complementary oligos, not less than 25 bp in length, which will contain your target sequence somewhere in the middle. It's a good idea to put some brand of sticky overhang (*Kpn* I, for example) on the ends so you can clone the site easily should the need arise.

Labeling is done with γ-^{32}P-ATP and T4 kinase (see protocol).

1. Following instructions from T4 kinase protocol, label each oligo separately in 20-25 ul reactions.

2. Combine the two reactions and boil 2 minutes; slow cool to RT (at least 10 min.).

3. Spin down for 3 sec, then remove unincorporated radiolabel by passing duplexed material through spin column (see protocol).

4. Count CPM.

The amount of label you use is up to you. Don't use less than 10K cpm per lane. If you use 100K cpm per lane, you can get "instant images" in 20 min. Overnight exposures will be good at RT without a screen and probably overexposed at -80° with a screen.

Setting up reactions:

1. Set up reactions in 25 ul.

2. The conditions which work best may vary with the protein and ought to be optimized. A reasonable starting point follows:

Final concentrations in binding reaction:

25 mM Tris HCl, pH 7.5
80 mM NaCl
35 mM KCl
5 mM MgCl2
1 mM DTT
100-250 ng dI-dC per reaction, as non-specific competitor.

3. Add probe and protein last. You will have to titrate to determine how much protein to use. 100 ng-2ug is the range to be in.

4. Playing with Mg and NaCl/KCl may improve binding. Addition of 5-10% glycerol (final conc.) and/or 1-2% Ipegal (formerly known as NP40, also final conc.) may enhance binding.

5. Mix by pipetting up and down. Incubate @ RT for 30min (Incubation time/temp. are subject to optimization).

6. Gently load all of reaction on EMSA gel.

EMSA Gels

EMSA GEL Makeup:

0.5 X TBE
5% Acryamide
2% Glycerol
Pour at least one hour before using.

1. Run in 0.5 X TBE buffer at RT, 100V, 2 hours (may have to run longer depending on probe length).
2. Transfer to Whatman, cover with saran wrap.
3. Dry @ 80° for 30 min, expose.