EMSA (gel shift) Protocol
(BLACK LAB)

TNT Translation
25 μl lysate
2 μl buffer
1 μl T7 pol
1 μl AA-met
1 μl RNAsin
18 μl dH_2O
48 μl per rxn

To 48 μl mix, add 2 μl plasmid (0.5 μg/μl).
To 5μl of that add 1μl ^{35}S Met.
To the rest add 2μl -LEU
Incubate 90 min at 30˚C

(MAY DO 1/2X REACTION IF NOT MUCH IS NEEDED)

To analyze ^{35}S Met labeled TNT products:
Run 10% acrylamide minigel via SDS-PAGE protocol.

To anneal oligos:
5 μl top strand (1 mg/ml)
5 μl bottom strand (1 mg/ml)
10 μl 2x anneal (100mM Tris 7.6; 10mM DTT; 20mM MgCl2; 2mM Spermidine)

85-90˚C 10min
65˚C 10min
37˚C 10min
RT 10min

To label probes, do a fill-in rxn (be sure to have some "G" overhangs)**:**
4 μl annealed oligo
5 μl NEB2 Buffer
4 μl 0.5 mM GTP
4 μl 0.5 mM TTP
4 μl ^{32}P-dCTP
4 μl ^{32}P-dATP
24 μl water
1 μl Klenow (5 U)
50 μl total volume

60 min at 37˚C

To purify filled-in probes:
Add 6μl 10% loading dye

Prepare 10% acrylamide, non-denaturing 0.5X TBE gel.
[Use thin spacers and combs]
12.5 ml 40% acrylamide (20% acryl: 1% Bis)
 2.5 ml 10x TBE
35.0 ml dH_2O
Add 500μl 10% APS, 20 μl TEMED

Prerun gel for 40 min at 150V
Load with duck-billed tips, run BPB half way down, 150V (~2 1/2 hrs)
Expose 1min
USE FILM TO LINE UP WITH GEL AND CUT OUT GEL SLICE CORRESPONDING TO LABELED FRAG
Place gel slice in 100μl dH_2O (dep. on the efficiency of labeling)
Allow for elution of probe, 37˚C O/N
Remove the eluted probe, spin down 10 min to get rid of any remaining gel bits
Count 2μl (usually of a 1:10 dilution) in scintillation fluid.

Gel Shift Rxns:
Prerun gel 2hrs 100V

Add components in this order:
dH_2O (to 18 μl final vol.)
2 μl 10X Binding buffer
1 μl dIdC (1mg/ml stock)
protein (10 μg extract, 2-5 μl lysate)
competitor or A/b

10 min RT
      Add 2 μl probe diluted to 10,000 cpm/μl
20-30 min RT
      (May add A/b after 5min for supershift)

Add 2μl of BPB-Glycerol dye (Maniatis)
Load with duck-billed tips
Run at constant voltage 150 V for ~3 hrs (BPB one inch from bottom)
Dry gel
Expose O/N -80˚C with intensifying screen

BUFFERS AND REAGENTS

10x Binding buffer
400 mM KCl
150 mM HEPES 9pH 7.9)
 10 mM EDTA
  5 mM DTT
50% Glycerol

Gel Shift Gel (6% acryl/ 0.5x TBE)
10.0 ml 30% Acrylamide (30% Acryl: 1% Bis)
 2.5 ml of 10X TBE)
37.5 ml dH_2O
600 μl 10% APS
 30 μl TEMED

30% Acrylamide Stock solution (30:1 Acrylamide:Bis) 500 ml
145 g Acrylamide
  5 g Bis-acrylamide
      Use mask/ fume hood. Filter – store at 4˚C wrapped in foil

40% Acrylamide (20:1 Acrylamide:Bis) 200 ml
76 g Acrylamide
 4 g Bis
Use mask/ fume hood. Filter – store at 4˚C wrapped in foil

10x Loading dye (for probe purif.)
10 ml 100% Glycerol
 2 ml 0.5 M EDTA pH 8
 Traces of BPB and XC

Buffer 0.5X TBE