Gel Shift Assay for Detection of TBP-DNA Complexes and Mot1 activity

6% Polyacrylamide Gel (14 cm X 16 cm X 0.2 cm)

Stock acrylamide (20% acrylamide/0.33% bis acryl)	10.5 ml
10 X TG buffer	3.5 ml
50% glycerol	1.75 ml
0.5 M DTT	35.0 µl
1 M magnesium acetate	0.35 ml
water	18.9 ml

35.0 ml total
de-gas, then add 0.3 ml 10% APS and 30 µl TEMED

Running buffer is 1 X TG buffer with 5 mM magnesium acetate
(60 ml 10 X buffer + 3 ml 1 M magnesium acetate + water: 600 ml final volume)

10 X TG buffer (500 mls)
15.0 g Tris
71.4 g glycine
1.9 g disodium EDTA

adjust pH to 8.3

Pre-run gel at 100 V in cold room while setting up the binding reactions.

5 X Binding Buffer (store at -20 C)

20% glycerol	400 µl 50%
20 mM Tris-Cl, pH 8	20 µl 1 M
300 mM KCl	300 µl 1 M
25 mM MgCl2	25 µl 1 M
500 mg/ml BSA	10 µl 50 mg/ml
0.025% Bromophenol Blue	25 µl 1%

220 µl water

A typical 20 µl binding reaction contains:

4 µl 5 X Binding Buffer
0.2 µl 100 mM DTT
1000 cpm radiolabeled DNA (final concentration usually about 0.5 nM)
0.2 µl 0.5 mg/ml poly [dG-dC] (Amersham 27-7910-02)
2.0 µl 1.0% Brij 58 (Sigma P-5884)
TBP (about 3 ng), Mot1 (0.3-3.0 µl from standard isolation; ~30 – 150 ng)
± 1 µl 500 µM ATP
water to 20 µl

Add water +/- ATP to eppendorf tubes. Make mix with binding buffer, DTT, poly dG-dC, DNA, Brij 58 and TBP (if it doesn't vary); dispense to tubes at room temperature. Add Mot1 as indicated. Mix gently by flicking with finger, quick spin. Incubate ~25 minutes at room temp and then load onto gel while it's running in the cold room. Load and run reactions at 160 V until dye is 1/2 to 2/3 of the way down (~20-80 minutes). Recirculation of buffer isn't necessary. Dry gel and expose to film overnight or phosphorimager screen.