Electrophoretic Mobility Shift Assay (EMSA: gel shift) for VSG ES promoter

Gel casting and running

Setup of apparatus:

1. Prepare polyacrylamide mix: 36.5 ml autoclaved water, 8.1 ml 30.8% polyacrylamide stock sol, 5.0 ml 5x TBE.

2. Add 300 µl ammonium persulfate, 100 µl TEMED; cast gel: polymerization occurs in 30 minutes.

3. Pre-run gel in 0.5x TBE for 30 min at 100V (RT: room temp).

4. When the pre-run is almost complete, start preparing the DNA binding reactions.

Binding reactions

5. Add the binding buffer, water, poly (dI.dC), DTT, and polyamines as a premix (aliquot subsequently).

6. Add the protein extract to the reaction and incubate for 5 min at RT.

7. Add DNA probe, incubate 10 min at RT before loading onto gel. Add loading dye to empty well to monitor electrophoresis (no dye in samples).

8. Run gel at 100–150V for 3-4 h at 4°C.

9. Attach gel to a piece of Whatman paper, dry it, expose 6 h to film or 2 h to Phosphorimager.