Gel Shift (EMSA) Conditions

Gel Shift Probe

H_2O	- 34.5µl	- incubate at R.T. 15 mins.
4.B	- 2.5µl	- add 20µl STE buffer
(30ng) DNA	- 3.0µl	- Nuctrap
NTP's	- 5.0µl	- add 12µl STE buffer
α^{32}pdATP	- 4.0µl	Final Vol. = 240µl
Klenow	- 1.0µl	
	50µl	- Count 1µl probe

EMSA Buffer Conditions

Crude Nuclear Extract Buffers:

Protease Inhibitors:

pepstatin A	0.5µg/ml
leupeptin	5µg/ml
Chymostatin	5µg/ml
antipain	5µg/ml
aprotinin	5µg/ml
benzamidine	5 mM

50 mM	KCl
4 mM	$MgCl_2$
20 mM	K_3PO_4 pH 7.4
1 mM	β-mercaptoethanol
20%	glycerol

- dialyse extract at 4°c for 30 mins in 500x volumes of extract buffer shown above.
- Store in small aliquot at -70°c. Use 5µg per rxn if possible.

Make 4 x EMSA buffer stock:

40 mM	HEPES pH 7.4	To 500µl add	- 10µl	BSA (20mg/ml)
20%	glycerol		- 2.5µl	PMSF (100mM)
4 mM	EDTA		- 2.0µl	DTT (1.0M)
		To each rxn 50µl	- 2.0µl	dIdC (1mg/µl) = 2µg/rxn
			- 2.0µl	KCl (1M) = 40 mM

- Add Components of each rxn to tube 1.5ml eppendorf and incubate at room temperature for 5 minutes
- add probe (2ng) and incubate further at R. temp for 15 minutes
- Run gel 160 Volts ..... Room 165V 1hr or 50V O/N

* Varying the concentration of non-specific competitor dIdC from 20ng to 2µg per rxn can allow the interaction of specific protein-DNA Complexes.