Electrophoretic mobility shift assay (EMSA) Protocol
11/29/11

This protocol is a modification of the lightshift Chemiluminescent EMSA kit (Thermo Scientific #20148) using biotin labeled oligos to detect the oligo/protein complex.

Complementary oligo annealing

- Mix equimolar amounts of each complementary oligo (50-100uM stocks of each), place in a heat block at 95 degrees for 5 minutes, then remove metal block and let cool down at RT

-quantify annealed oligo using a nanodrop or a Qubit; usual yields are 100-200ng/ul. We use 1ul of the unlabeled oligo and 1:300 dilution of the labeled oligo

- Aliquot the mixture and store at -80 degrees for long term and -20 degrees for working stock

Protein extracts

- HEK293T cells are transiently transfected with proteins of interest using lipofectamine 2000 (invitrogen #11668-027) and nuclear extracts were prepared using NE-PER nuclear and cytoplasmic extraction reagents (Thermo Scientific #78833)

-Nuclear and cytoplasmic fractions are aliquoted, snap frozen and stored at -80 degrees

Binding reaction

- Thaw protein extracts and buffers on ice and add each component to the binding reaction in the following order, keep reaction tube at room temperature:

Water	6 ul
10x bindinding buffer	2 ul
50% glycerol	4 ul
100mM MgCl2	1 ul
1ug/ul Poly (dI.dC)	1 ul
10 % NP-40	4 ul
Unlabeled oligo	1 ul
Protein extract	3 ul
Wait 10 minutes	
Biotin-labled oligo	1 ul
Total	20 ul

- Let the reaction go for another 30-60 minutes
- In the mean time prepare the gel for run

Gel Run

- pre-run a 5% native polyacrylamide gel in 0.5X TBE at 120V for 30 minutes in a Mini-PROTEAN tetra cell (Bio-Rad)
- add 3ul of 6X loading buffer to each reaction
- load all the reaction in the gel and run at 120V until the bromophenol blue dye runs 2/3 down the length of the gel
-Transfer gel on to a nylon membrane for 30 minutes at 100V in a Mini-PROTEAN tetra cell (Bio-Rad) in 0.5X TBE
-Crosslink membrane with a UV crosslinker
- Follow instructions for biotin detection on chemiluminescent nucleic acid detection module (Thermo scientific #89880) manual

Buffers:

10X binding buffer
250 mM Tris pH 7.5
800 mM NaCl
350 mM KCl
10 mM DTT

5 % native polyacrylamide gel

10x TBE	0.750 ml
30% acrylamide	2.5 ml
TEMED	13 ul
10 % APS	150 ul
Water	11.69 ml

6X loading buffer
15% Ficoll 400
0.25% Bromophenol Blue
0.25% Xylene cyanol
1X TBE