To: Dr. Erle Robertson

Shift-Western Protocol: ***(Reference: PNAS 90:2574, 1993.)***
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REAGENTS NEEDED: Nitrocellulose Membrane (Amersham)
DE81 Paper (Whatman)

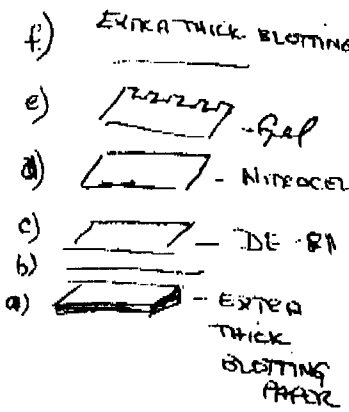
1. Binding reaction and EMSA is performed per lab protocol on a native polyacrylamide gel, however, all components of the binding reaction are 3 times the amount used in a regular gel shift is used). You do not want to overload your wells (i.e. use too much extract) as this will result in poor resolution of the bands.

2. Allow the "free probe" to run off the gel if a mini-gel is used. This will also allow for good separation of the different nucleoprotein complexes. If a mid-size gel is used, cut off the free probe area to avoid contamination of the transfer apparatus and also to minimize the amount of radiation handled.

3. Following the separation of nucleoprotein complexes transfer the protein-DNA complexes onto stacked nitrocellulose and anion exchange filters cut to the dimensions of the gel.
(NOTE: ***filter papers and blotting papers are pre-soaked in transfer buffer prior to use. Also, nitrocellulose and gel are equilibrated in transfer buffer prior to transfer(2-5 minutes); DE81 paper is <u>not</u> pre-soaked).***

TRANSFER BUFFER
(For 2 Liters)
Add: 48g Glycine
10.1g Tris. base

The sequence of layers (**<u>bottom to top</u>**) is as follows:



a. Pre-soak extra thick blotting paper (BIO-RAD) in transfer buffer and place on transfer apparatus.
b. Next, place a piece of 3MM Whatman filter paper.
c. Place the DE81 paper next, **however,** ***DO NOT PRE-WET!***
d. Place equilibrated nitrocellulose membrane.
e. Place the gel, taking note of orientation and alignment with DE81 paper and nitrocellulose. Remember, you want to be able to physically align the data from the autorad and the immunoblot eventually.
f. Place another piece of 3MM Whatman filter paper, followed by another pre-soaked piece of extra thick blotting paper.

4. Using BIO-RAD's Semi-Dry Transblot apparatus, transfer protein-DNA complexes for 30-42 minutes (.5-.7 of an hour) at 15 volts.

5. After transfer, nitrocellulose membrane is blocked and Western analysis procedures are continued as per protocol. DE81 paper is removed, dried for 2-5 minutes on gel dryer and radiolabelled DNA (positioned as on gel shift) bound to the DE81 paper is detected by autoradiography.