Preparation of DNA

1. In a micro centrifuge tube dissolve **500ug** of gel-purified double stranded annealed oligonucleotides to *ligation buffer #1* for a total volume of **75ul**.

2. Add **20ul** of *20mM ATP(pH 7)* and **5ul** of *T4 DNA ligase (10-30 Weiss units)*, to give a final volume of 100ul. Incubate mixture at 16 C for 4 hours.

3. DNA is Phenol-extracted*, precipitated with EtOH*, and dried in heto vacuum centrifuge.

4. Dissolve DNA in **100ul** of *autoclaved dd H2O* (Do not use TE buffer. It can interfere with coupling reaction)

*protocol details were not stated in Kadonaga, PNAS (1986) 83: 5889

Coupling DNA to CNBr-Sepharose

1. In 50ml screw cap tube dissolve DNA in **20ml** of *coupling buffer {0.1M NaHCO3 buffer(pH 8.5)* containing *0.5M NaCl}.*

2. In separate beaker wash and swell activated CNBr resin. Add **100ml** of *cold 1mM HCl* to **3g** of *CNBr resin* and mix for 5 min. Remove supernatant by **gentle** suction on Buchner funnel (**Do not** suction filter CNBr into a dry cake) use Whatman 1 qualitative filter paper to retain CNBr slurry. Transfer slurry back to beaker and repeat this washing step five more times with cold 1mM HCl. Resin will swell to ~12-15 ml.

3. Wash resin with **100ml** of ice *cold dd H2O* and suction filter supernatant.

4. Wash resin with **15ml** of *NaCO3/NaCl coupling buffer*, suction filter, and immediately transfer resin to the tube containing *coupling buffer/ DNA ligand.* Rinse filter paper with ~10ml of coupling buffer to collect remaining resin.

5. Mix DNA with resin for 2 hours at room temp. or over night at 4 C on shaker.

6. Suction filter DNA/resin into a fresh flask (save supernatant from this step for analysis to determine coupling efficiency) and rinse DNA/resin with **30ml** of *coupling buffer* to remove any unreacted DNA ligand

7. In another 50ml screw cap tube add resin to **20ml** of *0.2M glycine (pH 8.0)* and let sit at room temp for 2 hours or at 4 C overnight (16 hours).

8. Suction filter using **50ml** of *coupling buffer*: then filter using **50ml** of *0.1M sodium acetate/NaCl buffer (pH 4.0)*. Complete this wash cycle of high and low pH buffers four more times

9. Resin can be stored in *1.0M NaCl* at 4 C in a 50ml tube or immediately equilibrate in *Buffer A* and pack column

DNA Affinity Column

1. Mix **15ml** of *CNBr/DNA resin* is with **15ml** of *Buffer A* to make a 30ml 50% slurry. Pour slurry slowly into 2.5cm bio rad column using a glass rod held against the column wall to minimize introduction of air bubbles.

2. Wash column with **30ml** of *Buffer A*.

3. Dilute nuclear extract 1:4 in *Buffer A* and add **200ug** of *poly (dIdC)* to help reduce non specific binding in column. Allow mixture to stand for 10 min.

4. Pour diluted nuclear extract into column and collect flow through on ice and save. (step8)

5. Wash column with **75ml** of *Buffer A containing 100mM of NaCl*. Collect wash on ice and save. (step 8)

6.Elute proteins from column with a NaCl gradient. Collect into three fractions with each fraction in a separate 15ml tube.

1. *200mM NaCl/buffer A* - **7. 5ml x 2** = fraction 1
2. *500mM NaCl/buffer A* - **7. 5ml x 2** = fraction 2
3. *1M NaCl/buffer A* - **7. 5ml x 2** = fraction 3

Keep fractions on ice at all times.

7. Wash column with **30ml** of *1M NaCl/bufferA* collect on ice and save. (step8)

8. Take **1.5ml** aliquots of each fraction, 100mM& 1M washes, and flow through. Snap freeze at -140 C and process them for SDS Page gels.