Supporting Text

Materials and Methods.

Cell Fractionation. Nuclear and cytoplasmic extracts were prepared by using a protocol modified from Dignam and colleagues (1). Cells were washed with PBS and lysed on ice in hypertonic buffer with NP-40 [10 mM Hepes, pH 7.9/1.5 mM $MgCl_2$/10 mM KCl/0.5 mM DTT/0.1% NP-40 with Complete protease inhibitor (Roche)] for 5 min. The cytosolic fraction was harvested by centrifuging the lysate at 4,500 x *g* for 3 min and collecting the supernatant. The nuclear pellet was washed twice in hypertonic buffer without NP-40. Nuclear proteins were then extracted on ice in high-salt buffer [20 mM Hepes, pH 7.9/420 mM NaCl/1.5 mM $MgCl_2$/0.2 mM EDTA/0.5 mM DTT, 10% glycerol with Complete protease inhibitor (Roche)] for 30 min. The nuclear extract was harvested by centrifuging the lysate at 7,000 x *g* for 3 min and collecting the supernatant. Protein concentrations were determined using the BCA kit (Pierce) and 8 μg of protein extracts were loaded per lane and separated by SDS/polyacrylamide gel electrophoresis. Proteins were transferred onto Hybond-P membrane (Amersham), and the membrane blocked with 5% milk in PBS containing 0.1% Tween-20 (Sigma). The primary antibodies used were identical to those used for location analysis. The blots were developed with ECL (Amersham).

1. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. 1983 *Nucleic Acids Res.* **11,** 1475–1489.

Human Location Analysis Protocol **Version 1.7 11/10/03**

For self-printed PCR arrays

PROCEDURE

I. Cell cross-linking

Use $5x10^7 – 1x10^8$ cells (70-80% confluency for adhesion cells of 8-12 15 cm^2 plates or 175 cm^2 flasks) for each location analysis reaction.

Adherent cells:

1. Add 1/10 volume of fresh 11% formaldehyde solution to plates.
2. Swirl plates briefly and let them sit at room temperature for 10 min.
3. Add 1/20 volume of 2.5 M glycine to plates to quench formaldehyde.
4. Rinse cells twice with 5 ml 1X PBS. Harvest cells using silicon scraper.
5. Spin cells at 4,000 rpm for 10 min at 4°C.
6. Transfer cells to 15-ml conical tubes and spin 4,000 rpm for 10 min at 4°C.
7. Flash freeze cells in liquid nitrogen and store pellets at –80 °C.

Suspension cells:

1. Add 1/10 volume of fresh 11% formaldehyde solution to flasks.
2. Swirl flasks briefly and let them sit at room temperature for 20 min.
3. Add 1/20 volume of 2.5 M glycine to flasks to quench formaldehyde.
4. Spin cells at 2,500 rpm for 10' at 4°C.
5. Rinse cells with 100 ml 1x PBS and spin cells at 2,500 rpm for 10' at 4°C.
6. Rinse cells with 10 ml 1x PBS and transfer to 15ml conical tubes and spin 4,000 rpm for 10 min at 4°C.
7. Flash freeze cells in liquid nitrogen and store pellets at –80 °C.

II. Preblock and binding of antibody to magnetic beads

1. Wash 100 µl Dynal magnetic beads (per reaction) in 1 ml fresh BSA/PBS
2. Collect the beads by spinning at 3000 RPM for 3 minutes.
3. Wash beads in 1.5 ml BSA/PBS 2 times, collect the beads with the magnet.
4. Add 6-10 µg of Ab + 250 µl of PBS/BSA.
5. Incubate 4 hr to overnight on a rotating platform at 4°C.
6. Wash beads 3 times in 1.5 ml PBS/BSA.
7. Resuspend in 100 µl PBS/BSA.

REAGENTS AND CHECKPOINTS

11% formaldehyde solution

50 mls:	Final concentration
14.9 ml 37% formaldehyde	11%
1 ml 5M NaCl	0.1M
100 µl 0.5 M EDTA (pH 8)	1 mM
50 µl 0.5 M EGTA (pH 8)	0.5 mM
2.5 ml 1M Hepes (pH 8)	50 mM

2.5 M glycine

-Dissolve glycine in water with constant stirring.
-don't adjust pH

BSA/PBS Solution

100 ml:
10 ml 10x PBS
500 mg BSA
90 ml H_20
(good for at least a week)

III. Cell sonication

Note: Add protease inhibitors to all lysis buffers before use. If using a protease inhibitor cocktail tablet from Complete, dissolve one tablet in 2 ml H_2O for 25x solution. Store in aliquots at -20°C.

1. Resuspend each tube of cells in 5-10 ml of lysis buffer I. Rock at 4°C for 10 min. Then spin at 4,000 rpm in a table top centrifuge, 10 min at 4°C.
2. Resuspend each tube of cells in 5-10 ml of buffer 2. Rock gently at room temperature for 10 min. Pellet nuclei in tabletop centrifuge by spinning at 4,000 rpm, 10 min at 4°C.
3. Resuspend pellet in each tube in 3 ml buffer 3.
4. Sonicate the suspension in 15-ml conical tube with a microtip attached to Branson 250 sonifier, output setting at 5, constant power. Sonicate 12-15 times for 30 s, allowing the suspension to cool on ice for 1 min between pulses.
5. Add 1/10 volume of 10% Triton X-100. Transfer to 1.5-ml centrifuge tubes. Spin out debris 14,000 rpm, 10 min at 4°C.
6. Save 100 µl (or at least 1/50 vol) of cell lysate from each sample as input. Store at -80°C.

Lysis buffer 1 (LB1)

100 ml:	final concentration
5 ml 1M Hepes.KOH, pH 7.5	50 mM
2.8 ml 5M NaCl	140 mM
0.2 ml 0.5M EDTA	1 mM
10 ml glycerol	10%
5 ml 10% NP-40	0.5%
0.25 ml Triton X-100	0.25%

Lysis buffer 2 (LB2)

100 ml:	final concentration
4 ml 5M NaCl	200 mM
0.2 ml 0.5M EDTA	1 mM
0.1 ml 0.5M EGTA	0.5 mM
0.5 ml 2M Tris pH 8	10 mM

Lysis buffer 3 (LB3)

100 ml:	final concentration
0.2 ml 0.5M EDTA	1 mM
0.1 ml 0.5M EGTA	0.5 mM
0.5 ml 2M Tris·HCl, pH8	10 mM
2 ml 5M NaCl	100 mM
1 ml 10% sodium deoxycholate	0.1%
500 mg *N*-lauroyl sarcosine	0.5%

IV. Chromatin Immunoprecipitation

1. Add 100 µl antibody-prebound Dynal magnetic beads from step III.
2. Rock at 4°C overnight.

V. Washing, eluting, and reverse cross-linking

1. Transfer to centrifuge tubes, continue working in the cold room until step 6.
2. Use the magnetic stand to precipitate the beads.
3. Wash 4-8 times with 1 ml wash buffer
4. Wash once with 1 ml TE-plus-50 mM NaCl
5. Spin 3,000 rpm for 2-3 min and aspirate any residual TE buffer.
6. Add 100 µl of elution buffer.
7. Elute at 65°C for 10-15 min with brief vortexing every 2 min.
8. Spin down beads 14,000 rpm for 1 min.
9. Remove all 100 µl of supernatant.
10. Reverse cross-links overnight 65°C.
11. Thaw input, add 3 vol of elution buffer, and reverse cross-links overnight 65°C.

Wash buffer (RIPA buffer)

100ml:	final concentration
5ml of 1M Hepes (pH 7.6)	50 mM
200µl of 0.5M EDTA	1 mM
7 ml of 10% sodium deoxycholate	0.7%
10 ml of 10% NP-40	1%
10 ml of 5M LiCl or 2.12g powder	0.5 M

Elution buffer:
50 mM Tris, pH8
10 mM EDTA
1% SDS

Western analysis

-Elute 2nd time from beads with elution buffer and use a portion of elution to reverse x-link
-Check for precipitated protein by Western analysis

VI. RNase, Proteinase K

1. Add 1 vol of TE to IP and input fraction.
2. Add RNase A so final is 0.2 µg/µl (~5 µl/250 µl rxn). Incubate 37°C 1-2 hr.
3. Add proteinase K so final is 0.2µg/µl (~2.5 µl/250 µl rxn). Incubate 55°C 2 hr.
4. Extract once w/ 1 vol of phenol.
5. Extract once w/ 1 vol of phenol:chl:IA (made by mixing 1 vol of phenol w/ 1 vol of Chloroform:isoamyl alcohol).
6. Extract once w/ 1 vol of chl:IA.

4-6. Or instead extract 1x with 1 vol phenol:chl:IA using phaselock tubes

7. Add 30 µg (1.5 µl) of glycogen.
8. Add 5M NaCl so final is 0.2M (10 µl/250 µl rxn).
9. Add 2 vol of EtOH and incubate -80°C 30 min.
10. Spin and wash with 500 µl 75% EtOH.
11. Dry and resuspend pellets in 60 µl 10 mM Tris.HCl, pH 8. Save 5 µl of IP sample for checkpoints to the right. Normalize the input/wce fraction to 100 ng/µl using the Nanodrop.

VII. T4 Fill-in and blunt-end ligation

A. T4 DNA polymerase filled in

1. To 55 µl of IP sample, and 200 ng (=2 µl) of the normalized input/wce diluted to 55 µl add:
   - 11 µl 10X T4 DNA pol buffer
   - 0.5 µl BSA (NEB, 10 mg/ml)
   - 1 µl 10mM dNTP mix
   - 0.2 µl T4 DNA pol (NEB, 3U/µl)
   - up to 110 µl H_2O
2. Transfer sample to PCR tube and incubate 12°C 20 min (program 12/20).
3. Add 11.5 µl 3M NaAc and 0.5 µl (10 µg) glycogen.
4. Extract 1x with 120 µl phenol:chl:IA.
5. Precipitate with 250 µl EtOH.
6. Spin and wash with 500 µl 75% EtOH.
7. Dry pellet and resuspend in 25 µl H_2O.

B. Blunt-end ligation

1. Make at 4°C, 25 µl ligase mix per reaction:
   - 7.8 µl H_2O
   - 10 µl 5X ligase buffer (Gibco)
   - 6.7 µl annealed linkers (thaw at 4°C)
   - 0.5 µl T4 DNA ligase
2. Add mix to 25 µl of sample.
3. Incubate 12°C O/N, cover with foil.
4. Next day add 6µl of 3M NaOAc and 130 µl EtOH.
5. Spin and wash with 500 µl 75% EtOH.
6. Dry and resuspend pellet in 25 µl H_2O.

Fragment size verification

After sonication, the spread of size fragments can be checked by running 1 µl of WCE on a 1% agarose gel. Spread should extend no higher than 2 kb.

Gene-specific PCR

Check for IP enrichment specific for transcription factor by performing gene specific PCR with 2-3 µl of IP sample and a dilution series of input sample. Enrichment should be at least 2-fold over a nonspecific gene.

Oligos for blunt end ligation:

oJW102: 5'-GCGGTGACCCGGGAGATCTGAATTC
oJW103: 5'-GAATTCAGATC
See attached annealing protocol.

VIII. Ligation-mediated PCR

1. For PCR use 25 µl of IP sample and 25 µl input sample.
2. Make buffer mix per rxn:
   - 4.75 µl H_2O
   - 4 µl 10X Thermopol buffer (NEB)
   - 5 µl 2.5mM dNTP mix
   - 1.25 µl 40 µM oligo oJW102
3. Add mix to sample and start program CHIPCHIP: 55°C for 4'; 72°C for 3'; 95°C for 2'; 95°C for 30''; 60°C for 30''; 72°C for 1'; goto step 4 24 times; 72°C for 5'; hold at 4°C.
4. Midway to step 1 (55 °C → 4') add Taq mix:
   - 8 µl H_2O
   - 1 µl 10X ThermoPol buffer
   - 0.5 µl Taq (5U/µl)
5. Cleanup PCR product with Qiagen PCR column. Wash several times with PE buffer. Elute DNA with 60 µl EB buffer.

5. (Alternate) Or, add 25 µl of 7.5M NH_4OAc and 225 µl EtOH. Spin and wash pellet with 500 µl 75% EtOH. Redissolve in 50 µl H_2O.
6. Normalize [DNA] to 100 ng/µl.

Product size and amount verification

Remove 2 µl of PCR product and run it out on 2% agarose gel. Product sizes should be around 250-350 bp. Amount of IP PCR product should be roughly equal to input PCR product. Check the concentration using the Nanodrop, expecting ~50-100 ng/µl.

IX. Cy3-Cy5 random primer labeling

1. From the normalized IP and WCE PCR DNA above, label in duplicate, using 1 µg (=10µl of LM-PCR product) per rxn.
2. Add 10 µl water to above.
3. Add 20 µl of 2.5 X random primer solution (Invitrogen Bioprime labeling kit).
4. Boil 5 min in heatblock (use cap lock). Place tubes on ice. Incubate 5 min.
5. Add 5 µl 10X low T dNTP mix (1.2 mM dATP, dCTP, dGTP each and 0.6 mM dTTP).
6. Add 1 µl of cy5-dUTP to IP tube and 1 µl cy3-dUTP to input tube.
7. Add 1 µl of high concentration Klenow (40U/µl, Bioprime kit).
8. Incubate 37°C 1 hr (keep samples in dark as much as possible from here on).
9. Cleanup labeling rxn with Qiagen PCR purification column. Wash several times with PE buffer. Elute with 50 µl H_2O.
   8&9 (alternate). Incubate at 20°C 3-4 hrs and, instead of Qiagen, purify by adding 30 µl of 7.5M NH4OAc, and then re-ppt once by resuspending pellet into 100 µl of 2.5 M NH4OAc, and adding 3 vol EtOH to re-ppt.
10. Combine duplicate labeling samples.
11. Dry samples at 37°C (>10 min)
12. Dissolve in 15 µl H_2O.

Dye incorporation

Use Nanodrop microarray program to measure cy3 (550 nm) and cy5 (650 nm) incorporation. Ideally 100 pmol or more per slide, 50 pmol is the minimum.

X. Chip hybridization

A. Prehybridization.

1. Make 50 ml of prehybridization buffer per 4 arrays, take out 1 ml for hybe buffer.
2. Pour remaining prehybe buffer into Coplin jar. Preheat at 50°C 30 minutes.
3. Remove slides from vacuum and place them in the Coplin jar with prehyb buffer.
4. Incubate slides at 50°C for 45 minutes.
5. Wash slides with RO water and dry slides by spinning 1,000 rpm 1 min.
6. Fill two holes of bottom hybe chamber with water.
7. Place dried slides onto bottom chamber.

B. Probe preparation.

1. Combine cy5 labeled IP probes with cy3 labeled input probes.
2. Add 20 µg of human Cot-1 DNA (1 mg/ml) and 5 µl yeast tRNA (8 mg/ml).
3. Add 30 µl of 7.5M NH4OAc and 130 µl EtOH
4. Spin and wash with 500 µl 75% EtOH.

3-4 (Alt). Or, dry samples with speedvac.

5. Dry and resuspend pellet in 50 µl hybe buffer saved from prehybe step.
6. Denature probes 95°C for 5 min, spin briefly.
7. Incubate at 50°C until slides are ready (up to 5 min), spin briefly.
8. *Spot 47 µl of probes onto slides.
9. *Place cover slip (not at too steep an angle) and gently press down (from top end of slide) to evenly spread the probe solution so that it covers all the spots on the array.
10. Place the top hyb chamber and secure chamber tightly with metal brackets.
11. Place chamber in 50°C water bath for at least 16 hr.

Prehybridization solution (make fresh)

50ml:	final concentration
12.5 ml formamide	25%
12.5 ml 20x SSC	5x
0.5 ml 10% SDS	0.1%
0.1 g BSA	0.2%

(remove 1 ml of pre-hybe to be used as hybe buffer)

*Some find it easier to spot the hybe solution down the center of a cover slip then gently lower the array toward the coverslip. Place the coverslip on a tip box or other raised surface, spot the hybe solution in several drops down the center of the slip, invert a slide (array facing down) and carefully lower it toward the coverslip. When the array touches the liquid it will pull the slip against the array (don't drop the array onto the slip). Simply turn the array over so the coverslip is on top and place into the chamber as usual.

XI. Slide washing and scanning

A. Washing

1) Preheat Wash I to 50°C in Coplin jar (50ml per jar per 4 arrays)
2) Remove coverslip from slide and quickly immerse slide in of washing solution I.
3) Put jar on shaker and shake at 65 rpm for 5 min at room temperature.
4) Transfer slides to glass dish and rack containing washing solution II. Shake for 10 min.
5) Transfer slides to new glass dish with a new rack containing wash III (transfer slides only to minimize detergent carryover). Shake 1 min.
6) Repeat step 5 two more times. (3 x 1 minute washes, use new dish & rack each time)
7) Dry slides by spinning at 1,000 rpm for 1 min or by spraying with N_2 gas.
8) Store slides in the dark.

B. Scanning

1. Turn on Axon scanner and open GenePix Pro software.
2. Place slide upside down in scanner.
3. Prescan.
4. During prescan, adjust the cy3 channel (532 nm PMT voltage under hardware settings) to a background signal of ~100 or less by pointing the cursor to area where there is no spot and viewing the p (pixel) value on the side bar.
5. Set up the proper scanning window
6. Scan.
7. Adjust the cy5 channel (635 nm) so the intensity of the spot signals of both channels approximately match, using combination of techniques below
    a. Use histogram to overlap green and red graphs
    b. Intensity ratio around 1
    c. Toggle between 2 wavelength images to adjust by eye
8. When set, stop scan and rescan image
9. Save image file as multi-channels in TIFF format.
10. Load appropriate GPS template.
11. Align template over all spots.
12. Inspect all spots closely and adjust diameters of spots appropriately.
13. For spots that are bad, flag them as "flag not found."
14. Click to analyze and save results as .gpr files.

Washing solution I

100 ml:	final concentration
10 ml 20X SSC	2X
2 ml 10% SDS	0.1%

Washing solution II

300 ml:	final concentration
1.5 ml 20X SSC	0.1X
3 ml 10% SDS	0.1%

Washing solution III

1l:	final concentration
5 ml 20X SSC	0.1X

Linker oligonucleotide annealing

Mix the following:

1 ml 1 M Tris.HCl, pH 7.9
1.5 ml oligo oJW102 (40 μM)
1.5 ml oligo oJW103 (40 μM)

oJW102: GCGGTGACCCGGGAGATCTGAATTC

OJW103:
GAATTCAGATC

Aliquot into 50 or 100 μl fractions in 1.5-ml tubes.
Place in a 95^{o}C heat block for 5 mins.
Take heat block from heater and let it cool on the bench to room temperature.
Transfer the block to 4^{o}C and allow to stand overnight.
Store aliquots at -20^{o}C.