1. Materials

1.1 Chromatin-IP

See protocol Chromatin-IP

1.2 Amplification of Immunoprecipitated DNA Targets

- 5X sequenase buffer (USB#70775)
- Sequenase (13 U/ul) (USB #70775Y)
- primer A (GTTTCCCAGTCACGATCNNNNNNNNN, HPLC purified)
- primer B (GTTTCCCAGTCACGATC)
- BSA (10 mg/ml)
- DTT (0.1 M)
- dNTPs (25 mM)
- PCR purification kit (Qiagen)
- Platinum Taq polymerase (Invitrogen)
- DNase I

2. Methods

2.1 Chromatin-IP

See protocol chromatin-IP

2.2 Amplification of CHIPed DNA

1. Assemble the following components in preparation for random primed linear amplification (Amplify IgG, AcH3, and αTF):

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified DNA</td>
<td>10 µl</td>
</tr>
<tr>
<td>5X sequenase buffer (USB#70775)</td>
<td>4 µl</td>
</tr>
<tr>
<td>primer A (200 µM)</td>
<td>4 µl</td>
</tr>
<tr>
<td>GTTTCCCAGTCAGTCACGATCNNNNNNNNN, HPLC purified</td>
<td></td>
</tr>
<tr>
<td>Total Volume</td>
<td>18 µl</td>
</tr>
</tbody>
</table>
2. Cycling Conditions:
   a. 94 °C for 4 minutes
   b. snap cool on ice
   c. 10°C for 5 min.

3. During incubation for each reaction assemble the following components:

   Per Reaction
   - BSA (10 mg/ml)     0.2 µl
   - DTT (0.1 M)      1.0 µl
   - dNTPs (25 mM)    0.5 µl
   - Sequenase (1.3 U/µl dilute stock 1:10) 1.0 µl
                      2.7 µl

4. Add 2.6 µl of the cocktail to the reaction mixture and use the following cycling conditions:
   a. Ramped to 37° C over 9 min (1°C/20 sec) to anneal primer A
   b. 37° C for 8 min
   c. 95° C for 4 min
   d. snap cool on ice
   e. 10° C hold
   f. add 1.0 µl Sequenase (1.3 U/µl) to each sample
   g. 10° C for 5 min
   h. Ramped to 37° C over 9 min (1°C/20 sec)
   i. 37° C for 8 min
   j. repeat c through i 2X
   k. hold at 4° C

5. Purify DNA using PCR purification kit (Qiagen).

7. Amplify DNA by PCR using primer B (GTTCGCCAGTCACGATC) and Platinum Taq polymerase by assembling the following components:

   Purified DNA     20 µl
   10X PCR Buffer   10 µl
MgCl₂ (25 mM) 3 µl
dNTPs (10 mM) 4 µl
Primer B 4 µl
Water 57 µl
Platinum Taq 2 µl
Total Reaction Volume 100 µl

a. 95° C for 30 sec Denaturation
b. 40° C for 30 sec Annealing
c. 50° C for 30 sec Annealing
d. 72° C for 60 sec Elongation
e. cycle back 29X

8. Amplified DNA is purified using Qiagen PCR purification kit.
9. DNA size is determined by loading 0.5 µl onto 1.2% agarose gel with 100 bp ladder (expected size range 200-1000 bp).
10. DNA was quantified using nanodrop (50-100 ng/µl or 6-9 µg total expected recovery).
12. DNA fragmentation accomplished by DNase I treatment.

2.3. Labeling of the probe
Labeling was used with TdT and biotynylated ddATP.

2.4. Hybridization
The hybridization of the labeled DNA was performed as described in the Affymetrix® Chromatin Immunoprecipitation Assay Protocol.
(http://www.affymetrix.com/support/downloads/manuals/chromatin_immun_ChIP.pdf)