ChIP-Chip

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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:

Per Reaction	
Purified DNA	10 µl
5X sequenase buffer (USB#70775)	4 µl
primer A (200 µM) GTTTCCCAGTCACGATCNNNNNNNNN, HPLC purified	4 µl
Total Volume	18 µl

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 (GTTTCCCAGTCACGATC) and Platinum Taq polymerase by assembling the following components:

Purified DNA	20 µl
10X PCR Buffer	10 µl

$MgCl_2$ (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˚ 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)