Affymetrix® Chromatin Immunoprecipitation Assay Protocol

P/N 702238 Rev. 3

For research use only.
Not for use in diagnostic procedures.

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Overview

Chapter 1

Introduction

The Affymetrix® Chromatin Immunoprecipitation (ChIP) Assay is designed to generate double-stranded labeled DNA targets that identify sites of protein-DNA interactions or chromatin modifications on a genome-wide scale. This assay has been designed specifically for use with Affymetrix GeneChip® Tiling Arrays for ChIP-on-chip studies in order to study transcription factor binding sites, histone protein modifications, and other chromatin-protein interactions.

ChIP experiments can be used as a powerful tool to complement RNA transcription studies because they enable researchers to study the DNA-protein interactions that regulate gene expression. Following the protocol, cells are first fixed with formaldehyde to crosslink DNA to any associated proteins. The cells are then lysed and DNA is sheared into smaller fragments using sonication. Protein-DNA complexes are then immunoprecipitated with an antibody directed against the specific protein of interest. Following the immunoprecipitation, crosslinking is reversed, samples are protease-treated and the purified DNA sample is amplified using a random-primed PCR method. Subsequently, targets are fragmented and labeled to hybridize onto GeneChip® Tiling Arrays. By comparing the hybridization signals generated by an immunoprecipitated sample versus an antibody-negative or non-specific antibody control, the regions of chromatin-protein interaction can be identified.

Studies were performed at Affymetrix to evaluate the robustness and sensitivity of the ChIP assay; however, because of the variability associated with the quality and affinity of various antibodies against their intended targets, results may vary from one antibody to the next. The procedure outlined in this protocol describes all the necessary steps and reagents for fixing cells, fragmenting chromatin, immunoprecipitating sheared chromatin, amplifying and labeling precipitated DNA.

We would like to acknowledge Mark Biggin and Xiao-Yong Li of the Lawrence Berkeley National Lab for sharing their modifications to the ChIP protocol. We have incorporated their improvements to the amplification step (page 23) with their approval.

Chromatin Immunoprecipitation Assay Protocol Optimization

This protocol has been developed for use with GeneChip® Tiling Arrays. Exact protocol conditions will require optimization by each user due to the variability inherent in:

- Experimental Biology: cell types, proteins of interest, antibodies
- Assay conditions: DNA fragmentation, PCR conditions

To ensure success with this protocol, it is critical that users optimize the following steps in the ChIP protocol prior to performing microarray hybridizations. Additional information on the optimization steps are available throughout this protocol.

1. Sonication conditions of fixed cells.

   Some cells are resistant to sonication treatment. Micrococcal nuclease treatment may improve DNA shearing for some cell lines.

2. Antibody Qualification.

   Antibodies should be qualified for use with chromatin immunoprecipitation experiments. ChIP qualification information is available from www.chiponchip.org or directly from antibody vendors.

3. Antibody Titration.

   Antibody affinities and avidities can vary, so the amount of antibody may need to be titrated to achieve optimal sample enrichment.

4. PCR amplification of immunoprecipitated DNA.

   The optimal number of PCR cycles may require optimization to avoid saturation and ensure that the IP enrichment is maintained.

5. QPCR Positive Control.

   A QPCR control is recommended to test ChIP conditions. This test requires a known protein binding to a known DNA sequence. After performing ChIP with an antibody to the known protein, QPCR is used to verify that the known DNA binding elements are enriched in experimental vs. negative control samples. This QPCR test can also be used to ensure that the enrichment of experimental samples vs. control samples is maintained after IP column clean up.

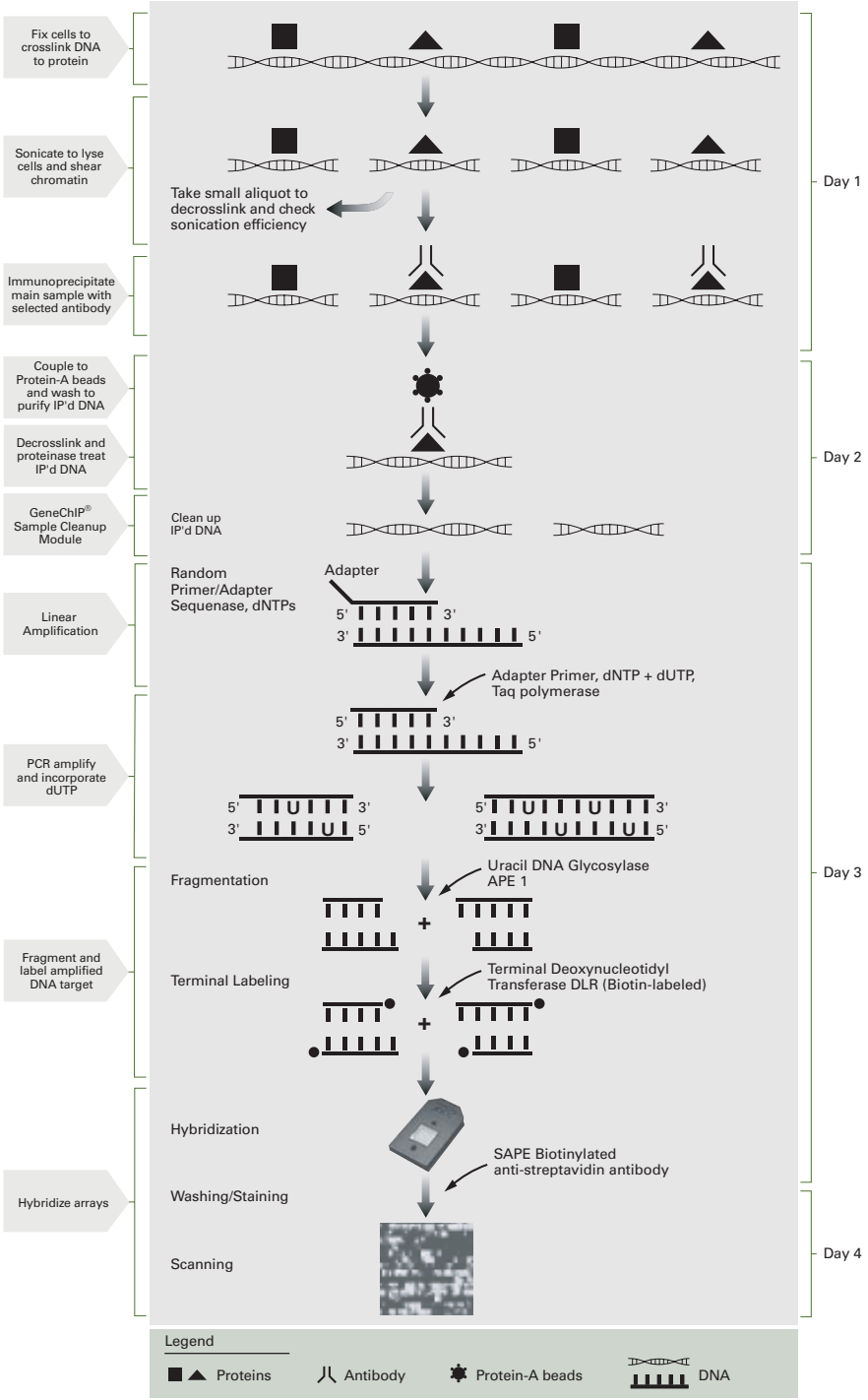


Figure 1.1
Chromatin Immunoprecipitation Assay Schematic

MATERIALS

Table 1.1
Materials Required

Material	Source	Part Number
Formaldehyde Solution (37%), 500 mL	Sigma-Aldrich	F8775
Glycine, 1 kg	Sigma-Aldrich	50046
Phosphate Buffered Saline (PBS) pH 7.4 (1X), liquid	Various	
IGEPAL® CA-630	Sigma-Aldrich	9036-19-5
Phenylmethanesulfonyl Fluoride Solution (PMSF), 250 mL	Sigma-Aldrich	93482
Microccocal Nuclease (MNase) (Optional)	USB	70196Y
EGTA (optional)	Sigma-Aldrich	E3889-100G
Protease Inhibitor Tablet	Roche	11873580001
Decrosslink and check sonication efficiency		
Proteinase K	New England BioLabs	P8102S
LiCl (8M), 500 mL	Sigma-Aldrich	L7026
Glycogen	Roche	10901393001
Immunoprecipitation		
Triton-X100 (non-ionic viscous liquid)	Roche	10789704001
Protein A Sepharose™ CL-4B	Amersham	17-0963-03
Antibody*	Various	

** NOTE: Antibody should be qualified for chromatin immunoprecipitation. See www.chiponchip.org for a list of qualified antibodies.*

Table 1.1 (Continued)
Materials Required

Material	Source	Part Number
PCR Amplification		
Sequenase™ Version 2.0 DNA Polymerase	USB	70775Y
Primer A: 200 µM GTTTCCCAGTCACGGTC$(N)_9$	Various	HPLC purified
Primer B: 100 µM GTTTCCCAGTCACGGTC	Various	HPLC purified
Taq Polymerase 5 U/µL	Various	
10X PCR Buffer	Various	
dATP 100 mM	Various	
dCTP 100 mM	Various	
dGTP 100 mM	Various	
dTTP 100 mM	Various	
dUTP 100 mM	Various	
BSA 20 mg/mL	Various	
DTT 0.1M	Various	
Wash Buffer		
Tris-HCl	Various	
EDTA	Various	
SDS, 100g	Sigma-Aldrich	71725
NaCl	Various	
Deoxycholate (sodium salt), 100 g	Sigma-Aldrich	D6750
$MgCl_2$, 1M	Various	
$CaCl_2$, 1M	Sigma-Aldrich	21115
Fragmentation and Labeling		
GeneChip® WT Double-Stranded DNA Terminal Labeling Kit, 30 Rxn	Affymetrix	900812

Table 1.1 (Continued)
Materials Required

Material	Source	Part Number
DNA Cleanup		
GeneChip® Sample Cleanup Module, 30 Rxn	Affymetrix	900371
ERC Buffer*, 85 mL	QIAGEN	1018144
Hybridization, Stain and Wash		
GeneChip® Hybridization, Wash, and Stain Kit	Affymetrix	900720
Control Oligonucleotide B2, 3nM	Affymetrix	900301

**NOTE: The GeneChip® Sample Cleanup Module includes 20 mL of cDNA Binding Buffer. In order to process 30 samples following the Affymetrix® Chromatin Immunoprecipitation Assay Protocol, additional cDNA Binding Buffer is required. This buffer should be purchased directly from QIAGEN. When purchasing the cDNA Binding Buffer from QIAGEN, please order "ERC Buffer," part number 1018144.*

BUFFERS

Table 1.2
Buffers

Lysis Buffer (Store at 4°C)
10 mM Tris-HCl (made from stock 1M Tris-HCl pH 7.5) 10 mM NaCl 3 mM $MgCl_2$ 0.5% IGEPAL 1 mM PMSF (add fresh)
Pre-IP Dilution Buffer (Store at RT)
10 mM Tris-HCl (made from stock 1M Tris-HCl pH 7.5) 10 mM NaCl 3 mM $MgCl_2$ 1 mM $CaCl_2$ 4% IGEPAL 1 mM PMSF (add fresh)
IP Dilution Buffer (Store at RT without protease inhibitors)
20 mM Tris-HCl (made from stock 1M Tris-HCl pH 8) 2 mM EDTA 1% Triton X-100 150 mM NaCl Protease Inhibitor Stock (add fresh)
Protease Inhibitor Stock
Prepare a 25X stock by dissolving 1 protease inhibitor tablet in 2 mL of nuclease-free water
ChIP Wash 1 (Store at RT)
20 mM Tris-HCl (made from stock 1M Tris-HCl pH 8) 2 mM EDTA 1% Triton X-100 150 mM NaCl 1 mM PMSF (add fresh)

Table 1.2 (Continued)
Buffers

ChIP Wash 2 (Store at RT)
20 mM Tris-HCl (made from stock 1M Tris-HCl pH 8) 2 mM EDTA 1% Triton X-100 0.1% SDS 500 mM NaCl 1 mM PMSF (add fresh)
ChIP Wash 3 (Store at RT)
10 mM Tris-HCl (made from stock 1M Tris-HCl pH 8) 1 mM EDTA 0.25M LiCl 0.5% IGEPAL 0.5% Deoxycholate (sodium salt)
Elution Buffer
25 mM Tris-HCl (made from stock 1M Tris-HCl pH 7.5) 10 mM EDTA 0.5% SDS

MISCELLANEOUS REAGENTS AND SUPPLIES

Table 1.3
Miscellaneous Reagents and Supplies

Material	Supplier	Part Number
Miscellaneous Reagents		
Absolute ethanol	Gold Shield Chemical Co.	
RNA-6000 Nano LabChip Kit	Agilent	5065-4476
Gel-Shift Assay (Optional)		
Novex XCell SureLock™ Mini-Cell*	Invitrogen	EI0001
TBE Gel, 4-20%,10 mm, 12 well*	Invitrogen	EC62252
5X Sucrose Gel Loading Dye	Amresco	E-274
10X TBE Buffer	Cambrex	50843
SYBR® Gold	Invitrogen	S-11494
10 bp DNA ladder and 100 bp DNA ladder	Invitrogen	10821-015 and 15628-019
ImmunoPure NeutrAvidin	Pierce	31000
PBS, pH 7.2	Invitrogen	20012-027
Miscellaneous Supplies		
1-2% Agarose Gells	Various	
1.5 mL RNase-free Microfuge Tubes*	Ambion	12400
1.5 mL Non-stick RNase-free Microfuge Tubes*	Ambion	12450
0.2 mL MicroAmp Reaction Tubes (8 tubes/strip)*	Applied Biosystems	N801-0580
MicroAmp Caps for 8 Strip Tubes	Applied Biosystems	N801-0535
Pipette for 25 mL*	VWR	53283-710
Pipet-Aid*	VWR	53498-103
Dolphin-nose Tubes	Costar (Corning)	3213
SpinX Columns	Costar (Corning)	8163
MicroSpin™ S-300 HR Columns	GE Healthcare	27-5130-01

Table 1.3 (Continued)
Miscellaneous Reagents and Supplies

Material	Supplier	Part Number
Instruments		
Rotating Benchtop Platforms	Various	
Branson Sonifier® S-450D	Branson Ultrasonics	101-063-590
Double Step Micro Tip Assembly	Branson Ultrasonics	101-063-212
NanoDrop® ND-1000*	Nanodrop Technologies	ND-1000
GeneChip® Hybridization Oven 640	Affymetrix	8001318
Eppendorf Centrifuge*	Eppendorf	5417C
Refrigerated Centrifuge with swing bucket rotor	Various	
Tube-Strip Picofuge™	Stratagene	400540
GeneChip® Fluidics Station 450 or 400	Affymetrix	00-0079
GeneChip® Scanner 3000 7G	Affymetrix	00-0073
GeneChip® Autoloader (Optional)	Affymetrix	90-0351
ABI GeneAmp PCR System 9700*	Applied Biosystems	N/A
Bioanalyzer 2100	Agilent	G2940CA
Heating Block*	VWR	13259-030
Pipette for 0.1 to 2 µL*	Rainin	L-2
Pipette for 2 to 20 µL*	Rainin	L-20
Pipette for 20 to 200 µL*	Rainin	L-200
Pipette for 100 to 1,000 µL*	Rainin	L-1000

**Or equivalent instrument/supplies.*

Chapter 2

Chromatin Immunoprecipitation Assay

Chapter 2

Procedure A: Prepare Cells

1. Grow enough cells for the number of immunoprecipitation (IP) reactions to be performed (usually 5 x 10^7 cells per IP for suspension cells, depending on IP efficiency). Prepare enough cells for two IP reactions. An antibody-minus (Ab- or mock IP) or non-specific IgG is recommended as a negative control using the same number of cells as the IP condition. The Ab- target would be treated identically to the experimental sample to serve as the "Control" group in the downstream two-sample analysis.
2. Use ~ 0.5 - 2 x 10^8 cells per IP. For example, grow 200 mL of 1 x 10^6 cells/mL for a total of 2 x 10^8 cells.

Procedure B: Fix Cells, Lyse, and Sonicate Whole Cell Extracts

DAY 1

NOTE **Centrifugation steps involving cells are best performed with a swing-bucket type rotor.**

ADHERENT CELLS

NOTE **End users may optimize the sequence of fixing and harvesting cells to minimize the degree to which cell physiology is disrupted.**

1. Add formaldehyde to the culture flask to a final concentration of 1% and incubate in a fume hood for 10 minutes.
2. Add 1/20 volume of 2.5 M glycine and incubate at room temperature (RT) for 5 minutes with gentle mixing.
3. Pour off formaldehyde media into an appropriate waste container and add enough ice-cold 1X PBS to cover the bottom of the flask to wash cells. Pour off PBS into a formaldehyde waste container and add enough PBS to cover bottom of flask.

4. Using a cell scraper, scrape off cells to re-suspend and check flask with microscope to ensure that most cells are re-suspended.
5. From here, go to Step 1 of the *Wash Cell Pellet* section below.

SUSPENSION CELLS

1. Fix cells by adding formaldehyde to a final concentration of 1% (add 5.5 mL of 37% formaldehyde to 200 mL of culture medium).
2. Incubate at room temperature (RT) in fume hood for 10 minutes, gently swirl 200 mL culture or invert tube containing 20 mL of adherent cells occasionally to mix cells.
3. Add 1/20 volume 2.5 M glycine and incubate at RT 5 minutes with gentle mixing to quench formaldehyde reaction. Perform remaining steps on ice.
4. Pellet cells at 4°C, (300-500*g*), 4 minutes and discard supernatant in formaldehyde waste.

WASH CELL PELLET

1. Wash pellet with 10 mL ice-cold 1X PBS to resuspend cells, and transfer to 15 mL tube.
2. Pellet cells at 4°C, (300-500*g*), 4 minutes and discard supernatant and repeat wash with ice-cold 1X PBS once.
3. Wash the pellet 3 times with 10 mL Lysis Buffer with fresh PMSF. Pellet cells at (300-500*g*) 5 minutes between washes.
4. Discard supernatant and proceed to the next step or flash freeze pellet and store at –80°C.
5. Resuspend the pellet in 1 mL pre-IP dilution buffer (add 60 µL PMSF) and bring final reaction volume to 1.5 mL with pre-IP dilution buffer.

6. Add to the tube:

Table 2.1

Component	Volume for 1 Rxn
100 mM PMSF	40 µL
25X Protease Inhibitor Stock	100 µL
Pre-IP Dilution Buffer	460 µL
20% SDS	100 µL
5 M NaCl	80 µL
Nuclease-free Water	220 µL*
Final Sample Volume Before Sonication	2.5 mL

*if using optional MNase, see details on page 19.

7. Sonicate sample to lyse cells and shear DNA to 100-1000 bp fragments. Some cell types (e.g., Jurkat) may require optional MNase treatment. See page 19 for details.

NOTE

Optimized shearing conditions are cell-type and instrument dependent. It is recommended that conditions are optimized with a single sample prior to scaling up procedures to multiple samples. Best sonication conditions at Affymetrix were achieved with a Branson Sonifier 450D (using a double-step microtip) set at 60% duty, 50% amplitude, 1 minute pulses with 1 minute rest. Both pulsing and resting steps were performed in an ice bath, 8 to 10 pulses total for HL-60 cells. Number of pulses may be dependent on cell density as well as cell type.

8. Aliquot the sonicated samples into two 1.5 mL microcentrifuge tubes, then microcentrifuge 14,000 rpm 10 minutes at 4°C to remove cellular debris.
9. Pool supernatants (from Step 8) in a 15 mL conical tube.

10. The sonication efficiency can be checked by taking an aliquot (100 µL) of this supernatant, de-crosslinking it (see Procedure C, below), and running the de-crosslinked DNA on a 1-2% agarose gel.
11. Divide the samples into aliquots equivalent to ~ 5 x 10^7 cells (1 IP), flash freeze and store at –80°C for later use or take straight through the IP.

Procedure C: Check Sonication Efficiency

1. Add 100 µL 10 mM Tris pH 8.0 to the 100 µL aliquot taken from the sonicated samples.
2. Add 2 µL Proteinase K (20 mg/mL) and mix well by vortexing.
3. Incubate 42°C for 2 hours, then 65°C for 6 hours to overnight (This step can be performed in a thermocycler.)
4. Clean-up using Affymetrix cDNA cleanup columns, from the GeneChip® Sample Cleanup Module, eluting with 20 µL Elution Buffer (see *Cleanup of Double-Stranded DNA* on page 57).
5. Load 100-500 ng of purified DNA sample on an agarose gel to check sonication efficiency. Typically, sheared DNA size ranges from 100-4000 bp, with the average size fragment between 200-1000 bp.

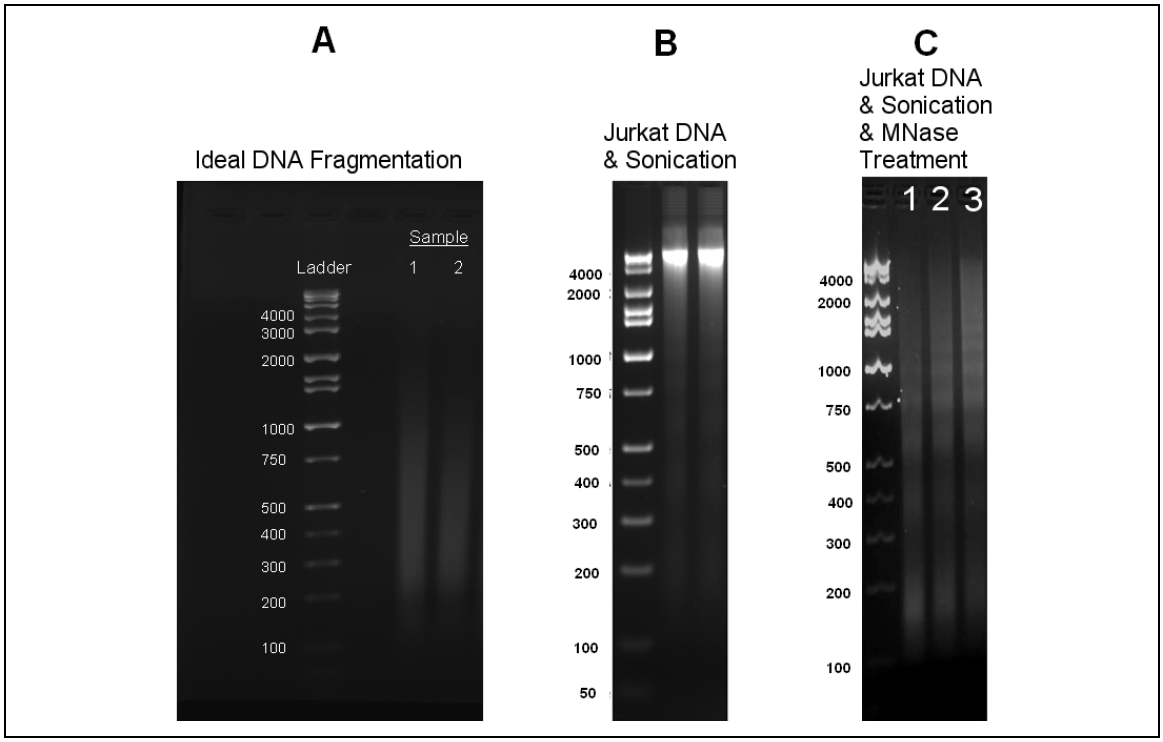


Figure 2.1
(A) Sheared DNA from HL-60 cells following 8 sonication pulses show the optimal size range for immunoprecipitation (~200-1000 bp with the majority of DNA fragments between 300-500 bp). Certain cell types may be more resistant to shearing by sonication and would require treatment with Micrococcal nuclease (MNase) to fragment chromatin. (B) Jurkat cells after 15 pulses of sonication show little fragmentation of crosslinked chromatin. (C) Fragmentation of Jurkat chromatin is achieved with MNase treatment. MNase enzyme concentration may have to be titrated based on cell type and density, **lane1**: 200U, **lane2**: 100U, **lane3**: 25U. The 'laddering' phenomenon seen with MNase treatment is common due to the specific cleavage of DNA by MNase between nucleosomes.

NOTE

Optional DNA Shearing Method
Micrococcal Nuclease Treatment

1. **Add appropriate units of MNase based on prior optimization of MNase to effectively shear crosslinked chromatin. This can range from 25 U to 200 U or more for each IP performed.**
2. **Incubate at 37°C, 10 minutes.**
3. **Add 30 µL 200 mM EGTA to stop the reaction.**

Procedure D: Incubate With Specific Antibody

1. If the sample (from Procedure B Step 11, on page 18) was frozen, thaw.
2. Transfer supernatant to a 15 mL tube and add 5 volumes of IP dilution buffer containing protease inhibitors (tablet from Roche, add before use).
3. Pre-equilibriate protein A Sepharose™ beads by washing 100 µL beads with 1 mL IP dilution buffer, pellet cells by centrifuging for 2 minutes at 2,000 rpm at 4°C in a microcentrifuge. Remove ~ 800 µL supernatant.
4. Pre-clear chromatin by adding 200 µL pre-equilibrated Protein A Sepharose beads.
5. Incubate on a rotating platform at 4°C for 30 minutes.
6. Centrifuge at 2,000 rpm for 2 minutes at 4°C in a swinging bucket rotor.
7. Transfer supernatant to a new 15 mL tube and discard beads.
8. Add 10 to 15 µg of antibody per IP. Usually, a negative control is performed using the same number of cells with a non-specific IgG or no antibody (mock IP) control.

NOTE **The amount of antibody to be added is dependent on quality, affinity, specificity, and type of antibody used. Users may have to titrate the amount of antibody used for each IP.**

9. Incubate on rotating platform at 4°C overnight (or for at least 3 hours at RT).

DAY 2

Procedure E: Immunoprecipitate and Wash

1. Pre-equilibrate protein A Sepharose™ beads by adding 1 mL IP Dilution Buffer and 200 µL beads for each IP'd sample. Centrifuge 2,000 rpm 2 minutes at 4°C.
2. Discard around 800 µL supernatant: save ~ 400 µL of beads in buffer at the bottom of the tube.
3. Transfer 400 µL beads to each sample.
4. Add PMSF to each tube sample (final concentration 1mM PMSF in final volume).
5. Incubate on rotating platform at RT for 1 to 3 hours.
6. Centrifuge at 2,000 rpm at 4°C for 4 minutes, and then discard supernatant.
7. Resuspend the pellet with 700 µL ChIP wash 1 (containing 1 mM PMSF added fresh), mix and transfer to spin-X column.
8. Incubate on rotating platform at RT for 1 minute.
9. Centrifuge at 2,000 rpm at RT for 2 minutes and discard flow-through.
10. Repeat steps 7–9.
11. Wash the beads with 700 µL ChIP wash 2 (containing 1 mM fresh PMSF).
12. Incubate on rotating platform at RT for 5 minutes.
13. Centrifuge at 2,000 rpm at RT and discard flow-through.
14. Wash the beads with 700 µL ChIP wash 3.
15. Incubate on rotating platform at RT for 5 minutes.
16. Centrifuge at 2,000 rpm at RT and discard flow-through.

17. Wash the beads with 700 µL TE (10 mM Tris-HCl pH 8, 1 mM EDTA).
18. Incubate on rotating platform at RT for 1 minute.
19. Centrifuge at 2,000 rpm at RT and discard flow-through.
20. Repeat steps 17 through 19.
21. Transfer the spin-X column with beads to a dolphin-nose tube.
22. Add 200 µL Elution Buffer to the column.
23. Incubate at 65°C for 30 minutes.
24. Centrifuge at 3,000 rpm at RT for 2 minutes.
25. Add 200 µL Elution Buffer to the column.
26. Centrifuge at 3,000 rpm at RT for 2 minutes. This 400 µL eluted sample is the "enriched" or "IP'd" sample.

Procedure F: Reverse Crosslinks

1. Add 5 µL Proteinase K (20mg/mL) per 100 µL of negative control or IP sample, mix well. (20 µL for 400 µL of eluted sample.)
2. Incubate in incubator at 65°C overnight.

DAY 3

Procedure G: Cleanup De-crosslinked Samples

1. Clean up samples using Affymetrix cDNA cleanup columns. Elute twice with 20 µL Elution Buffer (Appendix A, page 55). Total elution volume recovered is ~ 38 µL.

NOTE

2. IP efficiency can be checked at this stage in the protocol using polymerase chain reaction (PCR) and designing primer sets against regions that are known to be bound by the protein of interest and immunoprecipitated using the antibody being investigated. A significant increase or enrichment for the specific target should be observed for the IP condition compared to the Ab- control. Using quantitative real-time PCR, Affymetrix has routinely obtained >8-fold enrichment for IP samples compared to the Ab- samples.

Procedure H: PCR Amplify Immunoprecipitated DNA Targets

NOTE

Dilute Sequenase™ stock with Sequenase Dilution Buffer (included with enzyme) to 1.3 U/µL. Four microliters of this 1.3 U/µL working stock will be needed for each sample being amplified.

1. Use 10 µL of IP'd or negative control sample for initial round of linear amplification.

2. Set up first round reaction. Set up 1 reaction for single array products (e.g., Human Promoter 1.0R Array). Setup 3 reactions for multi-array sets (e.g., Human Tiling 2.0R Array Set).

Table 2.2

Component	Volume for 1 Rxn
Purified DNA	10 µL
5X Sequenase™ Reaction Buffer*	4 µL
Primer A (200 µM)†	4 µL
Total Volume	18 µL

**Included with enzyme.*
†Primer A: GTTTCCCAGTCACGGTC(N)$_9$ (HPLC purified)

3. Cycle conditions: Random priming.

A. 95°C for 4 minutes.

B. Snap cool samples on ice.

C. 10°C hold.

D. Prepare first cocktail.

Table 2.3
First Cocktail

Component	Volume for 1 Rxn
20 mg/mL BSA	0.1 µL
0.1 M DTT	1 µL
25 mM dNTPs	0.5 µL
Diluted Sequenase™ (1/10 from 13 U/µL stock)	1 µL
Total Volume	2.6 µL

E. Add 2.6 µL per sample.

F. Mix well by pipetting, and put the sample back in thermocycler block.

G. 10°C for 5 minutes.

H. Ramp from 10°C to 37°C over 9 minutes.

I. 37°C for 8 minutes.

J. 95°C for 4 minutes.

K. Snap cool on ice.

L. 10°C hold.

M. Add 1.0 µL of 1.3U/µL Sequenase™ to each sample.

N. 10°C for 5 minutes.

O. Ramp from 10°C to 37°C over 9 minutes.

P. 37°C for 8 minutes.

Q. Repeat from J) to P) for 2 more cycles.

R. 4°C hold.

4. For each IP, purify with Microspin S-300 HR (GE Healthcare) columns (2 columns per reaction) as follows:

A. Add 20 µL of 10 mM TE pH 8.0 to each reaction.

B. Spin 2 columns (A & B) at 3,000 rpm for 1 minute, discard flow-through.

C. Transfer reaction volume (~ 43 µL) to column A, while equilibrating column B with 300 µL of 10 mM Tris pH 8.0.

D. Spin both columns at 3,000 rpm for 1 minute, keep flow-through from column A (sample) and discard flow-through of column B (Tris buffer).

E. Transfer flow-through of column A to column B with new collection tube.

F. Spin at 3,000 rpm for 2 minutes.

G. Collect ~ 56 µL of first round purified DNA per reaction.

5. Prepare dNTP/dUTP mix.

NOTE

Prior to proceeding with the PCR amplification of immunoprecipitated DNA targets, prepare a dNTP mixture containing dUTP at the concentrations indicated below. Please note that this dNTP + dUTP mixture is only required for the PCR amplification reaction outlined in Table 2.4 and not in the Sequenase™ reaction setup in Table 2.3.
dCTP – 10 mM
dATP – 10 mM
dGTP – 10 mM
dTTP – 8 mM
dUTP – 2 mM
Store at –20°C.

6. PCR Mix Setup:

Table 2.4

Component	Volume for 1 Rxn
First-round DNA from Step 4	20 µL
10X PCR Buffer	10 µL
25 mM $MgCl_2$*	3 µL
10 mM dNTPs + dUTP	3.75 µL
100 µM Primer B†	4 µL
5 U/µL Taq Polymerase	2 µL
Nuclease-free Water	57.25 µL
Total Volume	100 µL

**Add $MgCl_2$ if using magnesium-free 10X PCR Buffer.*
†Primer B (GTTTCCCAGTCACGGTC)

7. Cycle conditions:

A. 15 cycles[1]

1) 95°C 30 seconds.

2) 45°C 30 seconds.

3) 55°C 30 seconds.

4) 72°C 1 minute.

B. 15 cycles[1]

1) 95°C 30 seconds.

2) 45°C 30 seconds.

3) 55°C 30 seconds.

4) 72°C 1 minute.

For every subsequent cycle add 5 seconds.
E.g., cycle 1: 60 seconds, cycle 2: 65 seconds, etc...

C. 4°C hold.

8. Check amplified DNA on 1% agarose gel.

[1] *Number of PCR amplification cycles may require optimization. QPCR can be used to evaluate enrichment of immunoprecipitated sample.*

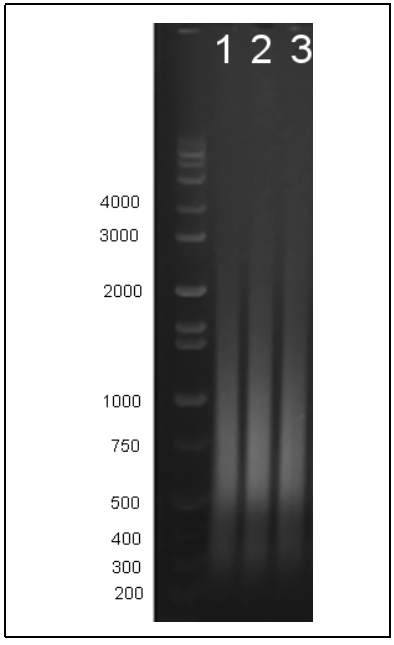


Figure 2.2
PCR-amplified ChIP targets from HL-60 cells immunoprecipitated with an Sp1 antibody. Replicate PCR reactions (lanes 1 to 3) were performed on the same IP sample and product sizes ranged from 200 bp to over 2 Kb but the actual product sizes may vary depending on original size of sheared chromatin.

9. Purify PCR samples with Affymetrix cDNA cleanup columns, provided in the GeneChip Sample® Cleanup Module, eluting twice with 20 µL of Elution Buffer.

10. Measure DNA using a NanoDrop or other UV-vis spectrophotometer. Normally, greater than 9 µg of amplified DNA is obtained from each reaction.

NOTE

Maintenance of IP enrichment post-amplification is crucial in obtaining good array results. QPCR should be performed to post-amplified samples to ensure that differences between the IP and Ab- samples are maintained. Primer sets can be designed for DNA regions that are known to be specifically immunoprecipitated using the antibody of interest.

Procedure I: Fragment Amplified Targets

1. Fragment the samples using the appropriate table below depending on what array type the target will be hybridized to.

Table 2.5
Fragmentation Mix for single arrays (e.g., Human Promoter 1.0R Array)

Component	Volume/Amount in 1 Rxn
Double-Stranded DNA	7.5 µg
10X cDNA Fragmentation Buffer*	4.8 µL
UDG, 10 U/µL*	1.5 µL
APE 1, 100 U/µL*	2.25 µL
Nuclease-free Water*	up to 48 µL
Total Volume	48.0 µL

** Available in GeneChip® WT Double-Stranded DNA Terminal Labeling Kit (P/N 900812)*

Table 2.6
Fragmentation Mix for multi-array sets (e.g., Human Tiling 2.0R Array Set)

Component	Volume/Amount in 1 Rxn
Double-Stranded DNA	9 µg
10X cDNA Fragmentation Buffer*	4.8 µL
UDG, 10 U/µL*	1.5 µL
APE 1, 100 U/µL*	2.25 µL
Nuclease-free Water*	up to 48 µL
Total Volume	48.0 µL

** Available in GeneChip® WT Double-Stranded DNA Terminal Labeling Kit (P/N 900812)*

2. Set up fragmentation mix according to either Table 2.5 or Table 2.6) Flick-mix and spin down the tubes.
3. Incubate the reactions at:
   - 37°C for 1 hour.
   - 93°C for 2 minutes.
   - 4°C for at least 2 minutes.
4. Flick-mix, spin down the tubes, and transfer 45 µL of the sample to a new tube.
5. The remainder of the sample is to be used for fragmentation analysis using a Bioanalyzer or agarose gel. Please see the Reagent Kit Guide that comes with the RNA 6000 LabChip Kit for instructions.

   If not labeling the samples immediately, store the fragmented DNA at –20°C.

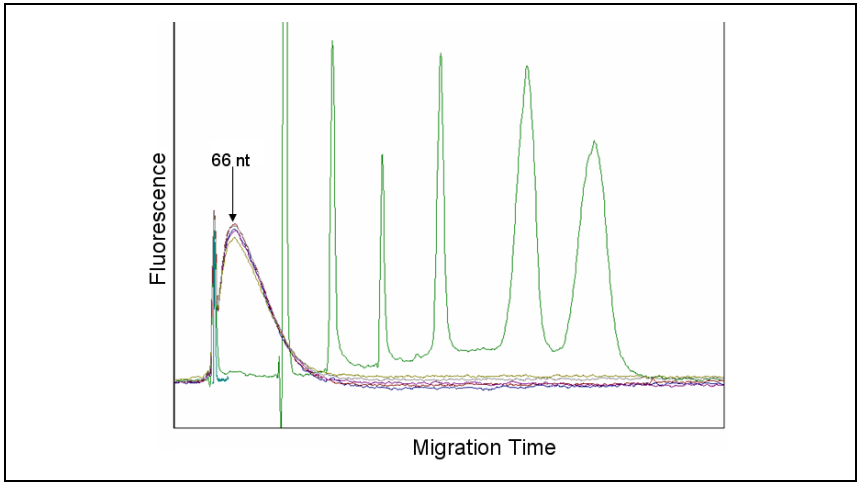


Figure 2.3
Bioanalyzer trace of fragmentation products following treatment of amplified ChIP targets with UDG and APE 1. Independently amplified Sp1 IP or Ab- samples from HL-60 cells were fragmented according to the protocol and products were analyzed on an Agilent Bioanalyzer with the RNA 6000 Nano LabChip Kit. Analyzing fragmented DNA on the RNA 6000 LabChip is recommended because it quickly assesses the degree and uniformity of the fragmented products.

Procedure J: Label Fragmented Double-Stranded DNA

1. Prepare the Double-Stranded DNA Labeling Mix as described in Table 2.7.

Table 2.7
Double-Stranded DNA Labeling Mix

Component	Volume in 1 Rxn
5x TdT Buffer*	12 µL
TdT*	2 µL
DNA Labeling Reagent, 5 mM*	1 µL
Total Volume	15 µL

** Available in the GeneChip® WT Double-Stranded DNA Terminal Labeling Kit (P/N 900812).*

2. Add 15 µL of the Double-Stranded DNA Labeling Mix to the DNA samples, flick-mix, and spin them down.
3. Incubate the reactions at:
   - 37°C for 60 minutes.
   - 70°C for 10 minutes.
   - 4°C for at least 2 minutes.
4. Remove 2 µL of each sample for gel-shift analysis (refer to the GeneChip® Whole Transcript (WT) Sense Target Labeling Assay Manual).

Chapter 3

Hybridization and Array Processing

Chapter 3

Procedure A: Hybridize Labeled Target on the Arrays

This Procedure requires the use of the GeneChip® Hybridization, Wash, and Stain Kit (P/N 900720). Alternatively, users may prepare their own hybridization mix using Table B.2 and either Table B.3 or Table B.4 in Appendix B.

1. Prepare the Hybridization Cocktail in a 1.5 mL RNase-free microfuge tube as shown in Table 3.1 and Table 3.2, below depending on what array type the target will be hybridized to.

Table 3.1
Hybridization Cocktail for single tiling arrays (e.g., GeneChip® Human Promoter 1.0R Array)

Component	Volume in 1 Rxn	Final Concentration or Amount
Fragmented and Labeled DNA Target	~ 60.0 µL*	~ 7.5 µg
Control Oligonucleotide B2	3.3 µL	50 pM
2X Hybridization Mix†	100 µL	1X
DMSO	14.0 µL	7%
Nuclease-free Water	up to 200.0 µL	
Total Volume	200.0 µL	

**This volume is 56 µL if a portion of the sample was set aside for gel-shift analysis.*

†Available in the GeneChip® Hybridization, Wash, and Stain Kit. If preparing buffers, see Appendix B for buffer composition.

Table 3.2
Hybridization Cocktail for use with serial hybridizations (e.g., GeneChip® Human Tiling 2.0R Array Set and GeneChip® Mouse Tiling 2.0R Array Set)

Component	Volume in 1 Rxn	Final Concentration or Amount
Fragmented and Labeled DNA Target	~ 60.0 µL*	~ 9.0 µg
Control Oligonucleotide B2	4 µL	50 pM
2X Hybridization Mix†	120 µL	1X
DMSO	16.8 µL	7%
Nuclease-free Water	up to 240.0 µL	
Total Volume	240.0 µL	

**This volume is 58 µL if a portion of the sample was set aside for gel-shift analysis.*

†Available in the GeneChip® Hybridization, Wash, and Stain Kit. If preparing buffers, see Appendix B for buffer composition.

2. Flick-mix, and centrifuge the tube.
3. Heat the Hybridization Cocktail at 99°C for 5 minutes. Cool to 45°C for 5 minutes, and centrifuge at maximum speed for 1 minute.
4. Inject ~ 200 µL of the specific sample into the array through one of the septa (see Figure 3.1 for location of the septa on the array). Save the remaining hybridization cocktail in –20°C for future use.
5. Place array in 45°C hybridization oven, at 60 rpm, and incubate for 16 hours.
6. After hybridization, remove the hybridization cocktail for future use.

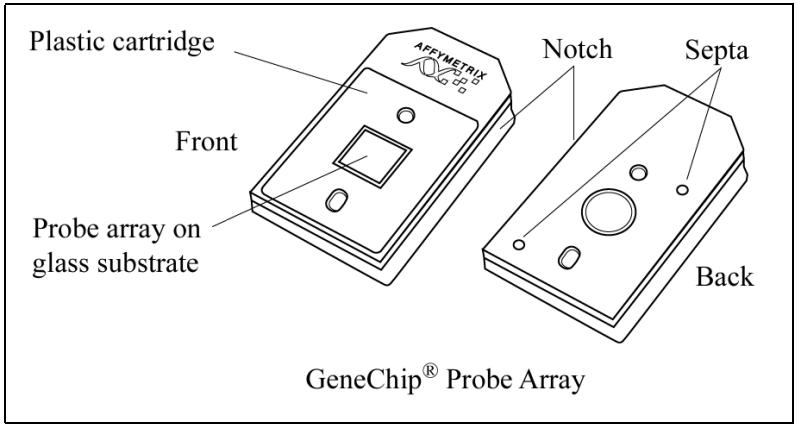


Figure 3.1
GeneChip® Probe Array

Chapter 4

Array Washing and Staining

Chapter 4

Procedure A: Enter Experiment Information

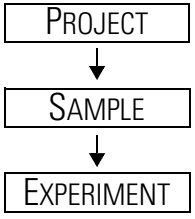
To wash, stain, and scan a probe array, an experiment must first be registered in GeneChip® Operating Software (GCOS). Please follow the instructions detailed in the "Setting Up an Experiment" section of the GCOS User's Guide.

The fields of information required are:

- Experiment Name
- Probe Array Type (For multi-array sets, please refer to the design number indicated on the array cartridge label.)
- Sample Name
- Sample Type
- Project

Sample templates, experiment templates, and array barcodes can also be employed in GCOS to standardize and simplify the registration process. Please see the *Affymetrix GeneChip® Operating Software User's Guide* (P/N 701439) for more information.

The Project, Sample Name, and Experiment Name fields establish a sample hierarchy that organizes GeneChip data in GCOS. In terms of the organizational structure, the Project is at the top of the hierarchy, followed by Sample Name and then Experiment Name.



Procedure B: Prepare the Fluidics Station

The GeneChip® Fluidics Station 450/250 or 400 is used to wash and stain GeneChip® Tiling Arrays. It is operated using GCOS.

Use the GeneChip® Hybridization, Wash, and Stain Kit (P/N 900720) or prepare buffers as indicated in Appendix B.

SET UP THE FLUIDICS STATION

1. Turn on the Fluidics Station using the toggle switch on the lower left side of the machine.
2. Select **Run → Fluidics** from the menu bar.

   The Fluidics Station dialog box appears with a drop-down list for selecting the experiment name for each of the fluidics station modules. A second drop-down list is accessed for choosing the Protocol for each of the fluidics station modules.

NOTE **Refer to the *Fluidics Station User's Guide* for instructions on connecting and addressing multiple fluidics stations.**

PRIME THE FLUIDICS STATION

Priming ensures that the lines of the GeneChip® Fluidics Station are filled with the appropriate buffers and the Fluidics Station is ready for running fluidics station protocols.

Priming should be done:

- when the Fluidics Station is first started.
- when wash solutions are changed.
- before washing, if a shutdown has been performed.
- if the LCD window instructs the user to prime.

1. To prime the fluidics station, select **Protocol** in the Fluidics Station dialog box.
2. Choose **Prime_450** for the respective modules in the Protocol

drop-down list.

3. Change the intake buffer reservoir A to **Wash Buffer A** (non-stringent wash buffer), and intake buffer reservoir B to **Wash Buffer B** (stringent wash buffer).
4. Select the **All Modules** check box, then click **Run**.

Procedure C: Wash and Stain Probe Arrays

After 16 hours of hybridization, remove the hybridization cocktail from the probe array and add to any remaining Hybridization Cocktail that was saved in Chapter 3, Procedure A. Fill the probe array completely with the appropriate volume of Non-Stringent Wash Buffer (Wash Buffer A), as described in Table 4.1 below.

Table 4.1

Format	Total Fill Volume
49	250 µL
64	250 µL
100	160 µL

NOTE

If necessary, at this point, the probe array can be stored at 4°C for up to 3 hours before proceeding with washing and staining. Equilibrate the probe array to room temperature before washing and staining.

This procedure takes approximately 90 minutes to complete.

FLUIDICS PROTOCOLS

Table 4.2
Fluidics Protocols for GeneChip® Tiling Arrays

	Fluidics Station 400 EukGE-WS2v5	Fluidics Station 450 FS450_0001
Post Hyb Wash #1	10 cycles of 2 mixes/cycle with Wash Buffer A at 30°C	10 cycles of 2 mixes/cycle with Wash Buffer A at 30°C
Post Hyb Wash #2	6 cycles of 15 mixes/cycle with Wash Buffer B at 50°C	6 cycles of 15 mixes/cycle with Wash Buffer B at 50°C
Stain	Stain the probe array for 10 minutes in SAPE solution at 35°C	Stain the probe array for 5 minutes in SAPE solution/Stain Cocktail 1 at 35°C
Post Stain Wash	10 cycles of 4 mixes/cycle with Wash Buffer A at 30°C	10 cycles of 4 mixes/cycle with Wash Buffer A at 30°C
2nd Stain	Stain the probe array for 5 minutes in antibody solution at 35°C	Stain the probe array for 5 minutes in antibody solution/Stain Cocktail 2 at 35°C
3rd Stain	Stain the probe array for 5 minutes in SAPE solution at 35°C	Stain the probe array for 5 minutes in SAPE solution/Stain Cocktail 1 at 35°C
Final Wash	15 cycles of 4 mixes/cycle with Wash Buffer A at 35°C. The holding temperature is 25°C	15 cycles of 4 mixes/cycle with Wash Buffer A at 35°C.
Holding Buffer	N/A - manual process	Fill the probe array with Array Holding Buffer.

- Wash Buffer A = non-stringent wash buffer
- Wash Buffer B = stringent wash buffer

Table 4.3

Format	FS450 Fluidics Protocol	FS400 Fluidics Protocol
49 e.g., Human Tiling 2.0R Array Set	FS450_0001	EukGE-WS2v5 and add Array Holding Buffer
64 e.g., Human Promoter 1.0R Array	FS450_0001	EukGE-WS2v5 and add Array Holding Buffer
100 e.g., *S. pombe* Tiling 1.0 FR Array	FS450_0002	Midi_euk2v3 and add Array Holding Buffer

WASH AND STAIN THE PROBE ARRAY ON FLUIDICS STATION 450

NOTE

If a Fluidics Station 450 instrument is unavailable, proceed with washing and staining with the appropriate FS400 fluidics protocol. Add Holding Buffer to the cartridge manually prior to scanning.

1. In the Fluidics Station dialog box on the workstation, select the correct experiment name from the drop-down **Experiment** list.

   The **Probe Array Type** appears automatically.

2. In the **Protocol** drop-down list, select FS450_0001 or FS450_0002 to control the washing and staining of the probe array.

3. Choose **Run** in the Fluidics Station dialog box to begin the washing and staining.

   Follow the instructions in the LCD window on the fluidics station.

   If you are unfamiliar with inserting and removing probe arrays from the fluidics station modules, please refer to the appropriate *Fluidics Station User's Guide* or *Quick Reference Card* (P/N 08-0093 for the FS-450/250 fluidics station).

4. Insert the appropriate probe array into the designated module of the fluidics station while the cartridge lever is in the down, or eject, position. When finished, verify that the cartridge lever is returned to the up, or engaged position.

5. Remove any microcentrifuge vials remaining in the sample holders of the fluidics station module(s) being used.
6. Follow the instructions on the LCD window. Place the following three vials (the microcentrifuge vials) into the sample holders 1, 2, and 3 on the fluidics station.
   - Place one vial containing 600 µL of SAPE Solution Mix/Stain Cocktail 1 in sample holder 1.
   - Place one vial containing 600 µL of Antibody Solution Mix/ Stain Cocktail 2 in sample holder 2.
   - Place one vial containing 800 µL Array Holding Buffer in sample holder 3.
   - Press down on the needle lever to snap needles into position and to start the run.

   The run begins. The Fluidics Station dialog boxes at the workstation terminal and the LCD window display the status of the washing and staining as they progress.
7. At the end of the run, or at the appropriate prompt, remove the microcentrifuge vials containing the stain solutions and replace with three empty microcentrifuge vials.
8. Remove the probe arrays from the fluidics station modules by first pressing down the cartridge lever to the eject position.
9. Check the probe array window for large bubbles or air pockets.
   - If bubbles are present, proceed to Table 4.4.
   - If the probe array has no large bubbles, it is ready to be scanned on GeneChip® Scanner 3000 7G. Pull up on the cartridge lever to engage washblock and proceed to Chapter 5, *Scanning* on page 49.

   If you do not scan the arrays right away, keep the probe arrays at 4°C and in the dark until ready for scanning.

   If there are no more samples to hybridize, shut down the fluidics station following the procedure outlined in the section, *Shut Down the Fluidics Station* on page 48.

Table 4.4

If Bubbles are Present
Return the probe array to the probe array holder. Engage the washblock by gently pushing up on the cartridge lever to the engaged, or closed, position. Follow the instructions on the LCD window. The fluidics station will drain the probe array and then fill it with a fresh volume of Array Holding Buffer. When finished, the LCD window displays 'EJECT CARTRIDGE.' Again, remove the probe array and inspect for bubbles. If no bubbles are present it is ready to be scanned. Proceed to Chapter 5, *Scanning* on page 49. If your attempt to fill the probe array without bubbles is unsuccessful manually drain the 1x Array Holding Buffer from the array, using a micropipette, and fill the array completely with a fresh aliquot of 1x Array Holding Buffer. Inspect the array and ensure that no bubbles are present. Excessive washing will result in a loss of signal intensity.

SHUT DOWN THE FLUIDICS STATION

1. After removing a probe array from the probe array holder, the LCD window displays the message **ENGAGE WASHBLOCK**.
2. If you are using the Fluidics Station 400, latch the probe array holder by gently pushing up until a light click is heard. Engage the washblock by firmly pushing up on the cartridge lever to the **ENGAGE** position.

   If you are using the Fluidics Station 450, gently lift up the cartridge lever to engage, or close, the washblock.

   - The fluidics station automatically performs a Cleanout procedure. The LCD window indicates the progress of the Cleanout procedure.
3. When the fluidics station LCD window indicates **REMOVE VIALS**, the Cleanout procedure is complete.
4. Remove the sample microcentrifuge vial(s) from the sample holder(s).
5. If no other arrays are to be processed, place wash lines into a bottle filled with deionized water.
6. Choose **Shutdown** or **Shutdown_450** for all modules from the drop-down **Protocol** list in the Fluidics Station dialog box. Click the **Run** button for all modules.

   The Shutdown protocol is critical to instrument reliability. Refer to the appropriate Fluidics Station User's Guide for more information.
7. After Shutdown protocol is complete, flip the ON/OFF switch of the fluidics station to the OFF position.

IMPORTANT !

To maintain the cleanliness of the fluidics station and obtain the highest quality image and data possible, the bleach protocol is highly recommended. Please refer to the *GeneChip® Fluidics Station 450/250 User's Guide* (P/N 08-0092) available at www.affymetrix.com.

Chapter 5

Scanning

Chapter 5

Scan

The GeneChip® Scanner 3000 7G is also controlled by GeneChip® Operating Software (GCOS). The probe array is scanned after the wash protocols are complete. Make sure the laser is warmed up prior to scanning by turning it on at least 10 minutes prior to use. If probe array was stored at 4°C, warm to room temperature before scanning. Refer to the GCOS online help and the appropriate scanner user's manual for more information on scanning.

WARNING ▲

The scanner uses a laser and is equipped with a safety interlock system. Defeating the interlock system may result in exposure to hazardous laser light.

You must have read, and be familiar with, the operation of the scanner before attempting to scan a probe array. Please refer to the the *GeneChip® Scanner 3000 Quick Reference Card* or user's manual.

HANDLING THE PROBE ARRAY

Before you scan the probe array, follow the directions in this section on handling the probe array. If necessary, clean the glass surface of the probe array with a non-abrasive towel or tissue before scanning. Do not use alcohol to clean glass.

Before scanning the probe array cartridge, apply Tough-Spots® label dots to each of the two septa on the probe array cartridge to prevent the leaking of fluids from the cartridge during scanning.

IMPORTANT !

Apply the spots just before scanning.

1. On the back of the probe array cartridge, clean excess fluid from around septa.
2. Carefully apply one Tough-Spots to each of the two septa. Press to ensure that the spots remain flat. If the Tough-Spots do not apply smoothly, that is, if you observe bumps, bubbles, tears, or curled edges, do not attempt to smooth out the spot. Remove the spot and apply a new spot. See Figure 5.1.

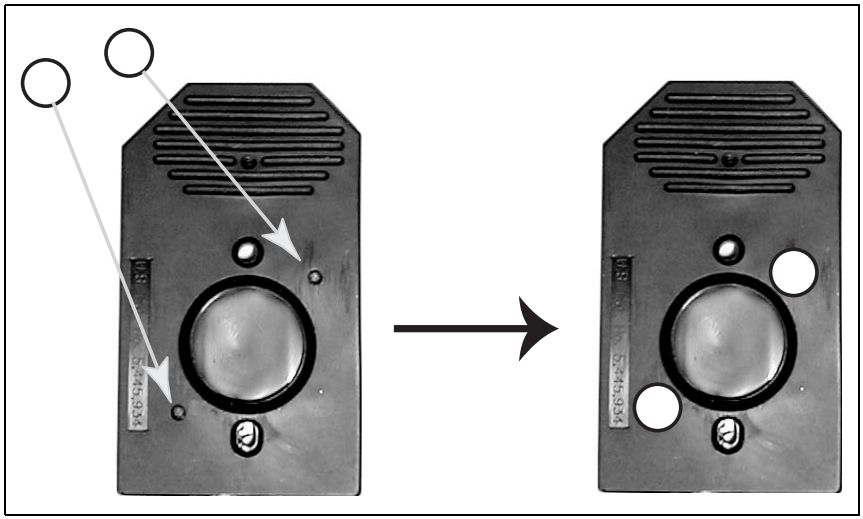


Figure 5.1
Applying Tough-Spots® to the probe array cartridge

3. Insert the cartridge into the scanner and test the autofocus to ensure that the Tough-Spots do not interfere with the focus. If you observe a focus error message, remove the spot and apply a new spot. Ensure that the spots lie flat.

SCANNING THE PROBE ARRAY

1. Select **Run → Scanner** from the menu bar. Alternatively, click the **Start Scan** icon in the tool bar.
   - The Scanner dialog box appears with a drop-down list of experiments that have not been run.
2. Select the experiment name that corresponds to the probe array to be scanned.

   A previously run experiment can also be selected by using the **Include Scanned Experiments** option box. After selecting this option, previously scanned experiments appear in the drop-down list.
3. Once the experiment has been selected, click the **Start** button.
   - A dialog box prompts you to load an array into the scanner.
4. Open the sample door on the scanner and insert the probe array into the holder. Do not force the probe array into the holder. Close the sample door of the scanner.
5. Click **OK** in the Start Scanner dialog box.
   - The scanner begins scanning the probe array and acquiring data. When **Scan in Progress** is selected from the **View** menu, the probe array image appears on the screen as the scan progresses.

Appendix A

Cleanup of Double-Stranded DNA

Appendix A

Cleanup of Double-Stranded DNA

This Procedure requires the use of the GeneChip® Sample Cleanup Module.

1. If not already done, add 24 mL of Ethanol (100%) to the cDNA Wash Buffer supplied in the GeneChip Sample Cleanup Module.
2. Add 5X volumes of cDNA Binding Buffer to sample, and vortex for 3 seconds.

NOTE

The GeneChip® Sample Cleanup Module includes 20 mL of cDNA Binding Buffer. In order to process 30 samples following the Affymetrix® Chromatin Immunoprecipitation Assay Protocol, additional cDNA Binding Buffer is required. This buffer should be purchased directly from QIAGEN. When purchasing the cDNA Binding Buffer from QIAGEN, please order "ERC Buffer," part number 1018144.

3. Apply the sample to a cDNA Spin Column sitting in a 2 mL Collection Tube (max capacity of column = 700 µL; if volume exceeds 700 µL, spin 700 µL at ≥ 8,000 x g for 1 minute, discard flow-through, and repeat).
4. Spin at ≥ 8,000 x g for 1 minute. Discard the flow-through.
5. Transfer the cDNA Spin Column to a new 2 mL Collection Tube and add 750 µL of cDNA Wash Buffer to the column. Spin at ≥ 8,000 x g for 1 minute and discard the flow-through.
6. Open cap of the cDNA Spin Column, and spin at ≤ 25,000 x g for 5 minutes with the caps open. Discard the flow-through, and place the column in a 1.5 mL collection tube.
7. Pipet recommended amount of cDNA Elution Buffer directly to the column membrane and incubate at room temperature for 1 minute. Then, spin at ≤ 25,000 x g for 1 minute.
8. Take 2 µL from each sample to determine the yield by spectrophotometric UV measurement at 260 nm, 280 nm and 320 nm:

   Concentration of Double-Stranded cDNA (µg/µL) = $[A_{260} - A_{320}]$ x 0.05 x dilution factor

   µg DNA = eluate in µL x DNA in µg/µL

Appendix B

Buffers and Solutions Required for Array Hybridization, Washing, and Staining

Appendix B

Buffers and Solutions Required for Array Hybridization, Washing, and Staining

Table B.1
Reagents for Hybridization, Wash, and Satin

Material	Source	P/N
GeneChip® Control Oligo B2, 3 nM	Affymetrix	900301
Acetylated Bovine Serum Albumin (BSA) solution (50 mg/mL)*	Invitrogen	15561-020
Herring Sperm DNA*	Promega	D1811
R-Phycoerythrin Streptavidin*	Molecular Probes	S-866
Goat IgG, Reagent Grade*	Sigma-Aldrich	I 5256
Anti-streptavidin antibody (goat), biotinylated *	Vector Laboratories	BA-0500
Water, Molecular Biology Grade*	Cambrex	51200
5M NaCl, RNase-free, DNase-free*	Ambion	9760G
MES hydrate SigmaUltra*	Sigma-Aldrich	M5287
MES Sodium Salt*	Sigma-Aldrich	M5057
EDTA Disodium Salt, 0.5M solution (100 mL)*	Sigma-Aldrich	E7889
DMSO*	Sigma-Aldrich	D5879
Surfact-Amps® 20 (Tween-20), 10%*	Pierce	28320
PBS, pH 7.2*	Invitrogen	20012-027
20X SSPE (3M NaCl, 0.2M NaH_2PO_4, 0.02M EDTA)*	Cambrex	51214
Tough-Spots®, Label Dots	USA Scientific	9185-0000

** Available in the GeneChip® Hybridization, Wash, and Stain Kit (P/N 900720).*

Table B.2
Buffers Required to be Prepared

12X MES Stock Buffer (1.22M MES, 0.89M [Na^+])
For 1,000 mL: 64.61g of MES hydrate 193.3g of MES Sodium Salt 800 mL of Molecular Biology Grade water Mix and adjust volume to 1,000 mL The pH should be between 6.5 and 6.7. Filter through a 0.2 µm filter. **IMPORTANT:** Do not autoclave. Store at 2°C to 8°C and shield from light. Discard solution if yellow.
2X Hybridization Buffer (Final 1X concentration: 100 mM MES, 1M [Na^+], 20 mM EDTA, 0.01% Tween-20)
For 50 mL: 8.3 mL of 12X MES Stock Buffer 17.7 mL of 5M NaCl 4.0 mL of 0.5M EDTA 0.1 mL of 10% Tween-20 19.9 mL of water **IMPORTANT:** Store at 2°C to 8°C and shield from light.
Wash Buffer A: Non-Stringent Wash Buffer (included in P/N 900720) (6X SSPE, 0.01% Tween-20)
For 1,000 mL: 300 mL of 20X SSPE 1.0 mL of 10% Tween-20 699 mL of water Filter through a 0.2 µm filter.

Table B.2 (Continued)
Buffers Required to be Prepared

Wash Buffer B: Stringent Wash Buffer (included in P/N 900720) (100 mM MES, 0.1M [Na^+], 0.01% Tween-20) **For 1,000 mL:** 83.3 mL of 12X MES Stock Buffer 5.2 mL of 5M NaCl 1.0 mL of 10% Tween-20 910.5 mL of water Filter through a 0.2 µm filter. **IMPORTANT:** Store at 2°C to 8°C and shield from light.
2X Stain Buffer (Final 1X concentration: 100 mM MES, 1M [Na^+], 0.05% Tween-20) **For 250 mL:** 41.7 mL of 12X MES Stock Buffer 92.5 mL of 5M NaCl 2.5 mL of 10% Tween-20 113.3 mL of water Filter through a 0.2 µm filter. **IMPORTANT:** Store at 2°C to 8°C and shield from light.
10 mg/mL Goat IgG Stock Resuspend 50 mg in 5 mL of 150 mM NaCl. Store at 4°C **IMPORTANT:** If a larger volume of the 10 mg/mL IgG stock is prepared, aliquot and store at –20°C until use. After the solution has been thawed it should be stored at 4°C. Avoid additional freezing and thawing.
1X Array Holding Buffer (100 mM MES, 1M [Na^+], 0.01% Tween-20) **For 100 mL:** 8.3 mL of 12X MES Stock Buffer 18.5 mL of 5M NaCl 0.1 mL of 10% Tween-20 73.1 mL of water Store at 2°C to 8°C and shield from light.

Table B.3
Hybridization Cocktail for single tiling arrays (e.g., GeneChip® Human Promoter 1.0R Array) if not using the GeneChip® Hybridization, Wash, and Stain Kit

Component	Volume in 1 Reaction	Final Concentration or Amount
Fragmented and Labeled DNA Target	~60 µL	~ 7.5 µg
Control Oligonucleotide B2	3.3 µL	50 pM
Herring Sperm DNA (10 mg/mL)	2.0 µL	0.1 mg/mL
Acetylated BSA (50 mg/mL)	2.0 µL	0.5 mg/mL
2X Hybridization Buffer	100 µL	1X
DMSO	14 µL	7%
RNase-free Water	up to 200.0 µL	
Total Volume	200.0 µL	

Table B.4
Hybridization Cocktail for use with serial hybridizations (e.g., GeneChip® Human Tiling 2.0R Array Set and GeneChip® Mouse Tiling 2.0R Array Set) if not using the GeneChip® Hybridization, Wash, and Stain Kit

Component	Volume in 1 Reaction	Final Concentration or Amount
Fragmented and Labeled DNA Target	~60 µL	~ 9.0 µg
Control Oligonucleotide B2	4.0 µL	50 pM
Herring Sperm DNA (10 mg/mL)	2.4 µL	0.1 mg/mL
Acetylated BSA (50 mg/mL)	2.4 µL	0.5 mg/mL
2X Hybridization Buffer	120 µL	1X
DMSO	16.8 µL	7%
RNase-free Water	up to 240.0 µL	
Total Volume	240.0 µL	

Preparing the Staining Reagents

Prepare the following reagents. Volumes given are sufficient for one probe array.

SAPE Stain Solution

Streptavidin Phycoerythrin (SAPE) should be stored in the dark at 4°C, either foil-wrapped or kept in an amber tube. Remove SAPE from the refrigerator and tap the tube to mix well before preparing stain solution. Do not freeze SAPE. Always prepare the SAPE stain solution fresh, on the day of use.

Table B.5
SAPE Solution Mix

Components	Volume	Final Concentration
2X Stain Buffer (see page 63)	300 µL	1X
50 mg/mL BSA	24 µL	2 mg/mL
1 mg/mL Streptavidin Phycoerythrin (SAPE)	6 µL	10 µg/mL
Molecular Biology Grade Water	270 µL	—
Total Volume	600 µL	

Mix well. The 600 µL of SAPE Solution Mix will be used for the 1st and 3rd stain.

NOTE

If using the Fluidics Station 400, after the first stain is done save the tube with the SAPE stain solution. Reuse the saved tube for the third stain.

Antibody Solution

Table B.6
Antibody Solution Mix

Components	Volume	Final Concentration
2X Stain Buffer	300.0 µL	1X
50 mg/mL BSA	24.0 µL	2 mg/mL
10 mg/mL Goat IgG Stock	6.0 µL	0.1 mg/mL
0.5 mg/mL biotinylated antibody	3.6 µL	3 µg/mL
Molecular Biology Grade Water	266.4 µL	—
Total Volume	600 µL	

Mix well. The 600 µL of Antibody Solution Mix will be used for the 2nd Stain.

Array Holding Buffer

To prepare the Array Holding Buffer, refer to Table B.2 on page 63.

Appendix C

Contact Information

Appendix C

Contact Information

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