



Agilent Technologies

AGILENT MICROARRAY GRANT APPLICATION SUPPORT

This guide offers an overview of Agilent's microarray and SureSelect product offerings with ordering details to assist researchers with the details of grant submission.

April 2, 2009

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Thank you for your interest in the Agilent Technologies, Inc. and good luck with your application.

Sincerely,

Agilent Technologies, Inc.

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General grant writing resources

- Agilent grant funding web page: www.agilent.com/chem/academia
- Information on Agilent microarrays: <http://www.chem.agilent.com/en-US/products/instruments/dnamicroarrays/pages/default.aspx>
- “All about Grants Tutorials” from the NIAID, NIH
<http://www.niaid.nih.gov/ncn/grants/default.htm>
- “Resources for Grant Applicants” from the NIH <http://grants.nih.gov/grants/oer.htm>
- “Overview of the Peer Review Process” from the CSR, NIH
<http://cms.csr.nih.gov/PeerReviewMeetings/CSRIRGDescription/>
- “Preparing Grant Applications” from the NCI, NIH
<http://deainfo.nci.nih.gov/extra/extdocs/apprep.htm>

Letter of Support

Dear Researcher,

On behalf of Agilent Technologies, I am pleased to offer our support for your applications involving the use of Agilent microarray products. Agilent will provide your choice of pre-designed or customized microarrays and any requested scientific design support. Design assistance is available through our expert scientists as well as our easy-to-use online array creation application tools. Agilent is committed to providing technical guidance including protocols, reagent and instrument recommendations, array processing assistance, and troubleshooting advice.

Given Agilent's flexible SurePrint inkjet printing process and rapid manufacturing, we anticipate that custom-designed microarrays will be fabricated and delivered in approximately 4-6 weeks after design finalization and order placement.

We look forward to our continued relationship as well as working with you on this exciting project. Please feel free to contact us if you have any questions or concerns.

Best Regards,

Chris Grimley
Senior Director of Marketing
Agilent Technologies
5301 Stevens Creek Blvd
Santa Clara, CA 95051

Experimental workflows

Agilent Technologies offers a wide spectrum of microarray based research tools to assist in research areas: gene expression (GE), miRNA, comparative genomic hybridization (CGH), DNA methylation, chromatin immunoprecipitation (ChIP-on-chip), and other emerging applications. Agilent produces tools to support entire experimental workflows from sample preparation, microarrays, scanning and hybridization hardware, and analysis software.

In addition to microarray applications, Agilent also produces the SureSelect Target Enrichment system which can be used to streamline next gen sequencing projects by focusing on specific sequencing regions of interest.

Target Enrichment For Next Gen Sequencing

The SureSelect Target Enrichment System uses an extremely efficient hybrid selection technique to target specific regions of interest to significantly improve the cost- and process efficiency of the sequencing workflow. With the SureSelect Target Enrichment System, only the genomic areas of interest can be sequenced, creating process efficiencies that reduce costs and allow more samples to be analyzed per study. The system leverages Agilent strengths: 1) proprietary SurePrint oligonucleotide synthesis of complex libraries consisting of ultra-long oligonucleotides greater than 100 bases in length; 2) custom library design fully integrated with eArray, Agilent's unique design tool; and 3) quality manufacturing processes that ensure the greatest reliability and consistency. The system can also easily be incorporated into an automated environment, further increasing process efficiencies, while minimizing total sample costs. As shown in **Figure 1**, the SureSelect Target Enrichment System workflow is solution-based and is performed in microcentrifuge tubes or microtiter plates. This format is more amenable to automation, and it can be scaled to meet the needs of larger sequencing projects, a limitation inherent in other commercially available methods of target enrichment.

Each target enrichment kit comes packaged with a mixture of custom SureSelect RNA oligonucleotides, or "baits," that are biotinylated for easy capture onto streptavidin-labeled magnetic beads, as well as buffers and blocking agents necessary for performing the capture process (**Figure 1**). To perform the capture, genomic DNA is sheared and assembled into a library format specific to the sequencing instrument utilized downstream. Size selection is performed on the library prior to capture and confirmed by a method such as electrophoresis on the Agilent Bioanalyzer. Size-selected libraries are then incubated with SureSelect baits for 24 hours. RNA bait-DNA hybrids are then "fished" out of the complex mixture by incubation with streptavidin-labeled magnetic beads and captured onto a strong magnet. After the beads have been washed, the RNA bait is then digested so that the only remaining nucleotide is the targeted DNA of interest. A few cycles of DNA amplification are performed at the end of the capture, and the targeted sample is then loaded onto the sequencing instrument.

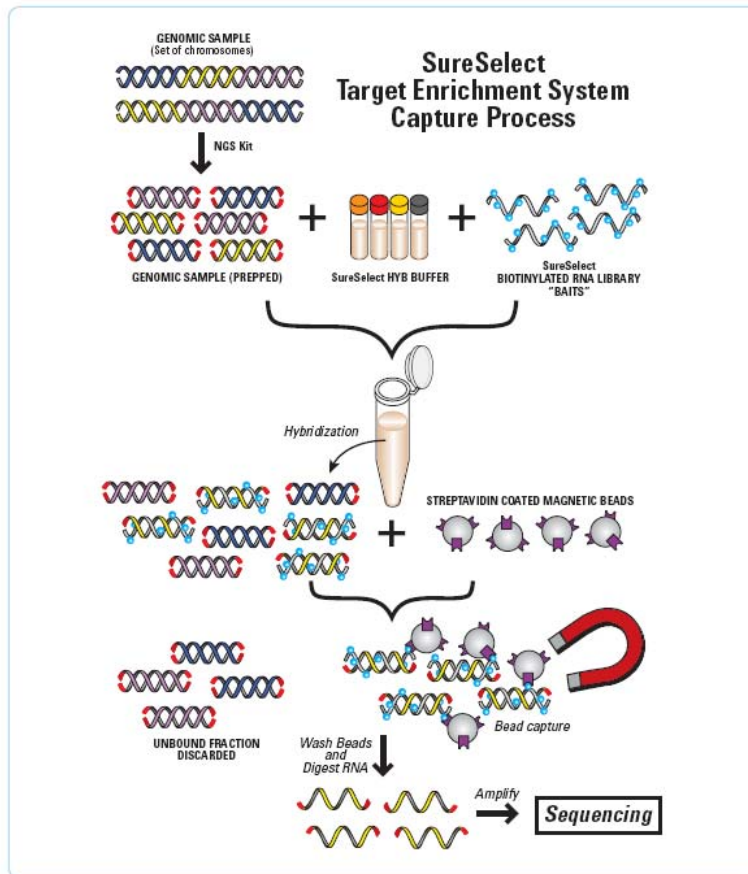


Figure 1. SureSelect Target Enrichment System Workflow

Differentiating Features of the SureSelect Target Enrichment System

1. The SureSelect Target Enrichment System is designed specifically for use with the Illumina Genome Analyzer.
2. The SureSelect Target Enrichment System is scalable and automatable platform for processing from 10- 1000's of samples.
3. The superior fidelity of Agilent oligonucleotide synthesis produces longer oligos for more efficient capture of sequence information and effective capture of sequences with mutations.
4. SureSelect is the only target enrichment product with eArray, an online design tool that simplifies experimental design.
5. SureSelect Target Enrichment System offers efficient capture of DNA containing mutations, with 1/10th the gDNA than competing products
6. SureSelect Target Enrichment System is the only target enrichment system that uses RNA/DNA hybridizations for the most efficient capture.

SureSelect Ordering Information

Description	Number of Reactions	Part Number
SureSelect Target Enrichment System Kit	10	G3360A
SureSelect Target Enrichment System Kit	25	G3360B
SureSelect Target Enrichment System Kit	50	G3360C
SureSelect Target Enrichment System Kit	100	G3360D
SureSelect Target Enrichment System Kit	250	G3360E
SureSelect Target Enrichment System Kit	500	G3360F
SureSelect Target Enrichment System Kit	1000	G3360G
SureSelect Target Enrichment System Kit	2000	G3360H
SureSelect Target Enrichment System Kit	5000	G3360J
SureSelect Human Chromosome X Exome Kit	5	G4459A

Citations to Agilent Target Enrichment

Gnirke A, et al. [Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing](#). Nat Biotechnol. 2009 Feb;27(2):182-9. Epub 2009 Feb 1.

Gene Expression Analysis

Agilent gene expression arrays have been cited in over 500 peer-reviewed publications (<http://opengenomics.com/publications.aspx>). Agilent produces and supports reagents, arrays, hardware and software for gene expression applications.

Gene Expression Analysis Method Description:

Quality assessment of total RNA is performed on the Agilent 2100 Bioanalyzer. 200ng total RNA is primed using an oligo-dT(T7) primer and converted to ds-cDNA using MMLV reverse transcriptase (RNaseH+) in a single reaction. This is followed by linear amplification using T7 RNA Polymerase to incorporate cyanine-CTP into the labeled aRNA target. In parallel, synthetic E1A Adenoviral sequences are spiked into the initial RNA and simultaneously labeled to serve as internal process controls for linearity, dynamic range, and log ratio fidelity. After column purification, labeled target is hybridized to the DNA microarray overnight with rotation at 65-C. Hybridized microarrays are washed, dried and scanned on the Agilent microarray scanner (G2565CA). Feature Extraction software is used for image analysis and the resulting statistics are analyzed via Agilent's GeneSpring software. Confirmation of differential expression changes is performed on the Agilent Mx QPCR instrument. The Agilent gene expression workflow is 1.5 days from RNA to data.

Differentiating Features of Agilent Gene Expression Arrays

1. Agilent gene expression arrays have a linear dynamic range >5logs compared to competitors at 3logs. This is critical for biomarker discover and uncovering subtle changes in gene expression that may have significant biological implications.
2. Agilent gene expression microarrays are the only commercial oligo arrays designed to be used in either 1-color or 2-color mode for flexibility in experimental design.
3. Agilent expression arrays are the only arrays that enable free web-based custom design for content iteration with no minimum order or design fees required.
4. Agilent gene expression arrays were found to provide the most accurate measurement detection of differential expression platforms measured by concordance and correlation to TaqMan (Nat.Biotech 24(9) Setp.2006, Supplemental Tables S12, S13).
5. Agilent gene expression arrays were found to offer the most sensitive detection of differential expression compared to competitive platforms (Nat.Biotech 24(9) Sept.2006, Fig.4, S7). This is critical for precious biological samples including LCM, blood, etc.

Agilent Gene Expression Reagents: Agilent gene expression arrays have the flexibility to either be processed using two color (Cy3, Cy5) or one color analysis. Quick Amp labeling kits are designed for amplification of eukaryotic polyadenylated samples. The FairPlay III kit uses random primers and is recommended for labeling prokaryotic samples.

Agilent Gene Expression Arrays: Agilent Gene Expression arrays are designed with specific 60-mer probes for each transcript. Arrays contain up to 44,000 unique probes. Arrays are multiplexed on 1x3" glass slides to facilitate processing of up to 8 samples in parallel. Agilent produces gene expression arrays to study human, mouse, rat, and a growing list of over 23 other organisms. See <http://www.chem.agilent.com/en-us/products/instruments/dnamicmicroarrays/pages/gp50628.aspx#microarrays> for the most current list of available gene expression arrays.

Gene Expression Data Analysis Software: GeneSpring GX provides powerful, accessible statistical tools for fast visualization and analysis of expression data. Designed specifically for the needs of biologists, GeneSpring GX offers an interactive desktop computing environment that promotes investigation and enables understanding of microarray data within a biological context. GeneSpring GX enables you to quickly and reliably identify targets of interest that are both statistically and biologically meaningful.

Required Materials for Gene Expression Array Experiments

Part Description	Part Number
Sample Isolation and QC	
Total RNA Isolation Mini Kit	5185-6000
Agilent RNA 6000 Nano Kit	5067-1511
Sample Labeling and Amplification	
One Color Eukaryotic Labeling	
Quick Amp Labeling Kit, one-color	5190-0442
One-Color RNA Spike-In Kit	5188-5282
Two Color Eukaryotic Labeling	
Quick Amp Labeling Kit, two-color	5190-0444
Two-Color RNA Spike-In Kit	5188-5279
Prokaryotic Labeling	
FairPlay III Microarray Labeling Kit	252009
Microarray Hybridization	
Gene Expression Hybridization Kit	5190-0404
Hi-RPM Gene Expression Hyb Kit, Large Volume	5190-0404
Microarray Wash	
Gene Expression Wash Buffer Kit	5188-5327
Analysis Software	
GeneSpring GX Standalone 1 year License - Academic	G3784AA

Citations to Agilent Gene Expression Arrays

MAQC Consortium, [The MicroArray Quality Control \(MAQC\) project shows inter- and intraplatform reproducibility of gene expression measurements.](#) Nat Biotechnol. 2006 Sep;24(9):1151-61.

miRNA Analysis

Agilent offers arrays, reagents, and software for profiling miRNAs in human, mouse, and rat. Arrays are updated to include content from Sanger mirBase updates. Arrays for miRNA profiling in other species can be designed using eArray.

Agilent miRNA Reagents: The Agilent miRNA Complete Labeling and Hyb Kit is for use with the Agilent miRNA Microarray System and provides a novel Cyanine 3-nucleotide along with array labeling and hybridization reagents. In combination with the miRNA

microarray probe design, the Cyanine 3-Cytidine bisphosphate (pCp) reagent selectively labels and hybridizes mature miRNAs.

Agilent miRNA Arrays: Agilent miRNA arrays offer accurate and consistent detection of small fold changes and good correlation with RT-PCR. Agilent miRNA arrays feature highly specific hybridization with low levels of cross-hybridization

miRNA Data Analysis Software: GeneSpring GX not only provides statistical tools to detect significant changes in miRNA abundance, but also integrates TargetScan miRNA gene target information to determine the biological consequences of the detected changes in miRNA abundance.

Differentiating Features of Agilent miRNA Arrays

1. Agilent miRNA profiling system offers a five orders of magnitude of linear dynamic range.
2. Detection of miRNAs in amounts as low as 10 zmol (~6000 molecules).
3. Agilent miRNA arrays Agilent miRNA array content is updated to include the latest information from Sanger mirBase database. Array updates are enabled by Agilent's flexible array manufacturing process.
4. Agilent is the only array manufacturer that offers online customization and design of miRNA arrays.

Required Materials for miRNA Array Experiments

Part Description	Part Number
Sample Isolation and QC	
Total RNA Isolation Mini Kit	5185-6000
Agilent RNA 6000 Nano Kit	5067-1511
Sample Labeling and Amplification	
miRNA Complete Labeling and Hyb Kit	5190-0456
Microarray Wash	
Gene Expression Wash Buffer Kit	5188-5327
Analysis Software	
GeneSpring GX Standalone 1 year License - Academic	G3784AA

Citations to Agilent miRNA Arrays

- Wang H, Ach RA, Curry B. Direct and sensitive miRNA profiling from low-input total RNA. RNA. 2007 Jan;13(1):151-9.
- Babak T. et al. Probing microRNAs with microarrays: Tissue specificity and functional interference. RNA (2004)
- Jackson A. et al. Expression profiling reveals off-target gene regulation by RNAi. Nature Biotechnology (2003)
- Cam H.P. et al. Comprehensive analysis of heterochromatin- and RNAi mediated epigenetic control of the fission yeast genome. Nature Genetics (2005)
- Lim L.P. et al. Microarray analysis shows that some microRNAs down regulate large numbers of target mRNAs. Nature (2005)

Comparative Genome Hybridization (CGH)

Array comparative genomic hybridization (aCGH) detects net copy differences between two samples of genomic DNA (eg. insertions, deletions, amplifications, etc) and provides higher resolution than traditional BAC and karyotype analysis (Genet Med 2008:10(4):278-289). It may be used in a range of applications ranging from analysis of solid tumors, genomic characterization of cell lines, postnatal cytogenetics, profiling of FFPE samples, and structural variation (CNV) analysis, etc. Agilent is the leading provider of aCGH workflow solutions including custom/catalog DNA microarrays, reagents, hybridization and scanning equipment, and data analysis software.

METHOD:

Agilent's array-based Comparative Genomic Hybridization (aCGH) application uses a "two-color" process to measure DNA copy number changes in an experimental sample relative to a reference sample. The type of sample used as a reference is a matter of experimental choice; however, many experimenters use normal commercial gDNA as a reference sample. Agilent supports two different labeling methods for genomic DNA: Enzymatic and ULS. For Enzymatic labeling, at least 0.5 µg (for 1x, 2x or 4x microarrays) or 0.2 µg (for 8x microarrays) of starting genomic DNA. You must use equal amounts of genomic DNA for both the experimental and reference channels in the restriction digestion step. The genomic DNA is digested by Alu1/Rsa1 and labeled by incorporation of cyanine-dUTP via Exo-Klenow and random primers. For the ULS labeling, utilizes Kreatech's Universal Linkage System (ULS), a non-enzymatic method to directly label genomic DNA with fluorescent dyes. In both methods, the labeled DNA is purified and free dye is removed using column purification options. After column purification, the labeled target is mixed with Cot-1 DNA, blocking agent, buffer and hybridized up to 40hr at 65C. Hybridized microarrays are washed, dried and scanned on the Agilent microarray scanner (G2565CA). Feature Extraction software is used for image analysis and the resulting statistics are analyzed via Agilent's DNA Analytics software. QPCR confirmation of changes may be performed using

Agilent's Mx QPCR instrument. Agilent's CGH protocol is amenable to both FFPE labeling and high-throughput sample processing.

Differentiating Features of Agilent CGH Arrays

1. Agilent CGH arrays have been shown to have the lowest baseline variation (noise) and highest response to single-copy losses and gains compared to competitive platforms (Genes Chromosomes and Cancer 47:697-711 (2008).
2. Agilent CGH arrays have been shown to have the lowest false positive/false negative rate and best sensitivity/specificity compared to competitive platforms (Cancer Res. 2007 Nov.1; 67(21):10173-80; Genomics 2007 Feb.1
3. Agilent aCGH arrays are the only oligo-CGH platform amenable for formalin-fixed (FFPE) samples.
4. Agilent aCGH arrays have been adopted at the standard in postnatal cytogenetics (Baylor, Emory, Signature press releases...etc).
5. Agilent's CGH and CNV microarrays enable high density precise copy number detection. With up to 1 million features per array and flexible custom array design options through eArray with the extensive database of > 24 M optimized probes, this versatile CGH/CNV platform identifies DNA copy number variations across the genome with exceptional sensitivity and reproducibility.
6. Agilent's CNV microarray was utilized in the largest CNV association study by the Wellcome Trust Case Control Consortium (PR 8/5/2008).

Required Materials for CGH Array Experiments

Part Description	Part Number
Sample Labeling and Amplification	
Enzymatic Labeling Genomic DNA Enzymatic Labeling Kit	5190-0449
ULS Labeling Genomic DNA ULS Labeling Kit	5190-0419
Genomic DNA Purification Module	5190-0418
Genomic DNA High-Throughput ULS Labeling Kit	5190-0450
Genomic DNA 96-well Purification Module	5190-0451
Microarray Hybridization	
Manual Hybridization Oligo aCGH/ChIP-on-chip Hybridization Kit (25 slides)	5188-5220
Oligo aCGH/ChIP-on-chip Hyb Kit, Large Volume (100 slides)	5188-5380
Tecan Robot Oligo aCGH Prehybridization Buffer (for Tecan HS Pro Station)	5190-0401
Microarray Wash	
Manual Hybridization Oligo aCGH/ChIP-on-chip Wash Buffer Kit	5188-5226
Tecan Robot Microarray Wash Buffer Additive (for Tecan HS Pro Station)	5190-0401
Analysis Software	
DNA Analytics	

Citations to Agilent CGH Arrays

- Gunnarsson R et al. Screening for copy-number alterations and loss of heterozygosity in chronic lymphocytic leukemia--a comparative study of four differently designed, high resolution microarray platforms. *Genes Chromosomes Cancer*. 2008 Au;47(8):697-711
- Greshock J et al. A comparison of DNA copy number profiling platforms. *Cancer Res*. 2007 Nov 1;67(21):10173-80.
- Stankiewicz P, Beaudet AL. Use of array CGH in the evaluation of dysmorphism, malformations, developmental delay, and idiopathic mental retardation. *Curr Opin Genet Dev*. 2007 Jun;17(3):182-92.
- Tonon G. et al. High-resolution genomic profiles of human lung cancer. *Proc. Natl. Acad. Sci. USA* (2005)
- Barrett M.T. et al. Comparative genomic hybridization using oligonucleotide microarrays and total genomic DNA. *Proc. Natl. Acad. Sci. USA* (2004)
- Brennan C. et al. High-resolution global profiling of genomic alterations with long oligonucleotide microarray. *Cancer Res*. (2004)
- Kidd et. al., Mapping and sequencing of structural variation from eight human genome. *Nature*. 2008 May 1; 453(7191): 56–64.
- J.R. Lupski et al. DNA duplication associated with Charcot-Marie-Tooth disease type 1A. *Cell*, 1991.
- J.A. Lee and J.R. Lupski, Genomic Rearrangements and Gene Copy-Number Alterations as a Cause of Nervous System Disorders. *Neuron* 2006. Perry et al., The Fine-Scale and Complex Architecture of Human Copy-Number Variation, *The American Journal of Human Genetics* (2008), doi:10.1016/j.ajhg.2007.12.010 de Smith AJ, et al *Hum Mol Genet*. 2007 Dec 1;16(23):2783-94. Epub 2007 Jul 31.
- Coe BP et al. Resolving the resolution of array CGH. *Genomics*. 2007 May;89(5):647-53. Epub 2007 Feb 2. PMID: 17276656

meDIP Methylated DNA Immunoprecipitation Arrays

meDIP is a Location Analysis method used to monitor methylation events in CpG Islands, areas around promoters, and other defined genomic regions such as those found in Human ENCODE. Agilent produces CpG Island Arrays, promoter arrays, and custom tiling arrays can also be constructed from almost any organism with available genomic sequence.

Differentiating Features of Agilent meDIP Arrays

1. Superior Microarray Performance. : Agilent 60-mer oligonucleotide probes and a convenient two-color labeling system delivers high sensitivity, accuracy, and greater reproducibility – these unique features allow sensitive measurements of DNA methylation events on a global scale.
2. Flexibility – Customization allows flexibility in research, a necessity in the evolving epigenetics field where content changes may be frequent. In addition, arrays can be customized in 1, 2, 4, or 8 arrays per slide formats, allowing researchers the desired level of focus.
3. End-to-end solution: Agilent provides a recommended, validated protocol, labeling kit, microarrays with all processing reagents, and an analysis package for DNA methylation research.
4. Optimal probe selection – Agilent carefully designs probes using stringent criteria giving optimal signal-to-noise. Our CpG Island probes were selected based on uniqueness and optimal hybridization properties. Repeat regions are masked to reduce nonspecific noise.

Required Materials for meDIP Array Experiments

Part Description	Part Number
Microarray Hybridization	
Manual Hybridization Oligo aCGH/ChIP-on-chip Hybridization Kit (25 slides)	5188-5220
Oligo aCGH/ChIP-on-chip Hyb Kit, Large Volume (100 slides)	5188-5380
Tecan Robot Oligo aCGH Prehybridization Buffer (for Tecan HS Pro Station)	5190-0401
Microarray Wash	
Manual Hybridization Oligo aCGH/ChIP-on-chip Wash Buffer Kit	5188-5226
Tecan Robot Microarray Wash Buffer Additive (for Tecan HS Pro Station)	5190-0401
Analysis Software	
DNA Analytics	

Citations to Agilent meDIP Arrays

- Kron K. et al. Discovery of Novel Hypermethylated Genes in Prostate Cancer Using Genomic CpG Island Microarrays. PLoS ONE (2009)
- Hahn M.A. et al. Methylation of polycomb target genes in intestinal cancer is mediated by inflammation. Cancer Research (2008)
- Hatada I et al. Astrocyte-specific genes are generally demethylated in neural precursor cells prior to astrocytic differentiation. PLoS ONE (2008)
- Rausch T.A. et al. High-resolution mapping of DNA hypermethylation and hypomethylation in lung cancer. Proc Natl Acad Sci (2008)
- Shen, Y. et al. X-inactivation in female human embryonic stem cells is in a nonrandom pattern and prone to epigenetic alterations. Proc Natl Acad Sci (2008)

ChIP-on-Chip (Chromatin Immunoprecipitation-on-chip)

ChIP-on-chip pairs chromatin immunoprecipitation (ChIP) with microarrays (chip) to analyze how regulatory proteins interact with the genome of living cells. ChIP-on-chip (also known as Location Analysis or LA) provides insight into key mechanisms of methylation, histone modification, as well as DNA replication, modification, and repair. High density arrays can be used to precisely determine the genomic location where a regulatory protein is bound.

Regulatory proteins bind to genomic DNA to control chromosome replication and gene activity, thereby functioning as switches in the regulatory circuitry of cells. This network of circuits is uncharted in many instances and its understanding will aid researchers and companies in identifying new target genes and therapeutics capable of modulating these pathways. Combining the new information gained from ChIP-on-chip studies with the wealth of already available gene expression data will help speed and focus both disease research and drug discovery.

Differentiating Features of Agilent ChIP Arrays

1. Superior Microarray Performance: Agilent 60-mer oligonucleotide probes and a convenient two-color labeling system delivers high sensitivity, accuracy, and greater reproducibility – these unique features allow sensitive measurements of weak- and infrequent-binding events for ChIP-on-chip.
2. Versatile user-defined microarray formats – Inherent flexibility of Agilent microarrays with up to 244K customizable features per slide in 1, 2, 4, or 8 arrays per slide format -- choose from over 10 species to customize your ChIP-on-chip experiments.
3. Agilent probe advantage – Average probe spacing is specifically optimized for ChIP-on-chip methods; repeat regions are masked to reduce noise and probes are filtered against homology.

Required Materials for ChIP Array Experiments

Part Description	Part Number
Microarray Hybridization	
Manual Hybridization	
Oligo aCGH/ChIP-on-chip Hybridization Kit (25 slides)	5188-5220
Oligo aCGH/ChIP-on-chip Hyb Kit, Large Volume (100 slides)	5188-5380
Tecan Robot	
Oligo aCGH Prehybridization Buffer (for Tecan HS Pro Station)	5190-0401
Microarray Wash	
Manual Hybridization	
Oligo aCGH/ChIP-on-chip Wash Buffer Kit	5188-5226
Tecan Robot	
Microarray Wash Buffer Additive (for Tecan HS Pro Station)	5190-0401
Analysis Software	
DNA Analytics	

Citations to Agilent ChIP-on-chip arrays

- Lee T.I. et al. Control of Developmental Regulators by Polycomb in Human Embryonic Stem Cells. *Cell* (2006)
- Boyer L.A. et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* (2006)
- Pokholok D.K. et al. Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* (2005)
- Boyer L.A. et al. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* (2005)
- Liu, X. et al. Yamanaka factors critically regulate the developmental signaling network in mouse embryonic stem cells. *Cell Research* (2008)
- Ferrari, R. et al Epigenetic Reprogramming by Adenovirus e1a. *Science* (2008)
- Agilent ChIP-on-chip Product Note
<http://www.chem.agilent.com/scripts/LiteraturePDF.asp?iWHID=41959>

Custom Microarrays Designed with eArray

eArray is a web based application that enables the creation of custom microarrays using Agilent's SurePrint in-situ oligonucleotide synthesis microarray platform for CGH, CHIP, Gene Expression and other emerging applications. eArray is free and there are no design fees associated with custom microarray design and manufacture.

<https://earray.chem.agilent.com/earray/>

Differentiating Features of Agilent Custom Arrays

1. eArray is the only free online interactive tool to design and order custom microarrays.
2. There are no design or consulting fees for Agilent custom arrays.
3. Agilent custom microarrays can be easily iterated using the flexible eArray design tool.
4. Quality: Arrays designed via eArray are produced with the same manufacturing and QC process as standard Agilent arrays.

Agilent Microarray Scanning and Hybridization Hardware

Agilent Microarray Scanner – Declaration of feature uniqueness

We are pleased to confirm that the Agilent G2565CA Microarray Scanner features a number of characteristics that make this product unique on the market. These unique characteristics are: An industry leading detection limit of 0.05 chromophores per square micron enabled by a patented method of scanning the microarray (US6902112, US6592036, US6371370)

- A dynamic autofocus feature that keeps the microarray surface in focus during the scan (US6486457)
- Internal calibration circuitry that ensures that the scanner is calibrated scan-to-scan, day-to-day, and month-to-month. (US6583424, US6740871, US6956203)
- A method of simultaneous scanning that enables both red and green channels to be scanned in 8 minutes at 5µm resolution and less than 20 minutes at 2µm resolution with low channel crosstalk of less than 0.1% (US6320196)
- A proprietary noise reducing optical design for consistent, high sensitivity performance (US6590689, US6222664)
- An extended dynamic range of over 105 for the detection of a broad range of signals on microarray (US7057185)
- Note: patents referenced not intended to be an exhaustive list of intellectual property covering the Agilent scanner.

Agilent DNA Microarray Hybridization Oven Features

- Oven designed to easily accept the Agilent rotator rack which holds in place up to 24 Agilent hybridization chambers during the hybridization incubation period
- Chambers, rotator rack, and oven constructed with rugged stainless-steel
- Oven includes drip pan, adjustable feet, locking pin, and power cord

- Available in 110V, or 220V configurations
- Oven rotation speed controlled from 5 and 20 RPM
- Hybridization Chambers covered under US Patent 6258593
- Hybridization Oven, Chambers, and Rotator manufactured specifically and exclusively for Agilent Technologies.

For information on instrument service: Agilent Services
<http://www.chem.agilent.com/Scripts/PCol.asp?lpage=137>.

Feature Extraction Software

Agilent's Feature Extraction 10.5 Image Analysis Software reads and processes microarray image files in an automated process and is an essential software tool for use with the Agilent Microarray Scanner. The software automatically finds and places microarray grids, rejects outlier pixels, accurately determines feature intensities and ratios, flags outlier pixels, and calculates statistical confidences. More information on Feature Extraction software is available:

<http://www.chem.agilent.com/Scripts/PDS.asp?IPage=2547>

Agilent SurePrint Inkjet Array Printing

Agilent Microarrays are produced using a proprietary ink jet printing process. This mask-less printing process is extremely flexible and produces exceptionally high quality oligos. The highly efficient oligo synthesis enables longer probe lengths

Agilent employs a seven-step microarray manufacturing process with many quality control checkpoints throughout the entire workflow. The diagram below shows the phases of the SurePrint array printing process

Step 1. Individual microarrays are group printed on large glass wafer sheets. These wafers are coated with a surface that makes a strong bond with both the wafer and the nucleic acids to be printed. Agilent microarrays are printed on high-quality glass that meets strict standards for uniform thickness and contains a smooth surface free from aberrations. The activated monolayer substrate coat used for nucleotide binding is rigorously controlled for de-lamination resistance, uniformity, and uniform spotting characteristics. In cases of problematic slide quality issues such as surface roughness, curvature, and warp, Agilent Microarray Scanners, unlike many other 3rd party scanners, can compensate.

Step 2. Standard phosphoramidite chemistry is used to produce oligo probes directly on the glass slide, allowing standard probe lengths of 60 nucleotides (**Figure 1**). Oligo synthesis reagents are carefully inspected for quality and purity and thoroughly tested for guaranteed uniform concentration and reaction specifics before entering the manufacturing process.

Step 3. The prepared wafers and nucleic acids combine together through a carefully orchestrated printing process. Multiple real-time quality control feedback mechanisms occur during the entire process - cameras visually inspect the presence, size, shape, and

position of each individual printed feature. The Agilent printing process is virtually identical to that of a standard inkjet printer, with solutions of the four nucleotides - A, T, C and G -deposited in lieu of conventional ink. The probe oligos are synthesized directly on the glass wafer by application of a precise amount of an individual base to a spot, followed by nucleotide bond synthesis, washing, and activation to bind the next nucleotide. For standard Agilent catalog microarrays, this process is repeated 60 times, but longer probe lengths are possible. The printer head never touches the wafer surface at any point during the process, resulting in highly uniform probe spots.

Step 4. Following completion of printing, the printed DNA is permanently bonded to the wafer surface, and surrounding surfaces around the features are deactivated. This last step helps reduce high background by minimizing the surface's ability to indiscriminately bind sample during hybridization. 5 of 13

Step 5. The microarrays printed on large glass wafer sheets are cut into individual 1" x 3" slides and bar-coded for identification. Agilent uses a high-quality two-step laser-scribing and dicing process to precisely machine the individual slides. This precision minimizes rough edges that can interfere with hybridization and scanning, and ensures centered alignment of the active microarray surface (**Figure 2**).

Step 6. Release of each print run depends upon a rigorous QC evaluation. Test microarrays from each run are hybridized with both control and reference targets to ensure that >95% of the experimental features are present, hybridizable, extractable, and uniformly printed (**Figure 3**).

Step 7. Microarray batches that pass the stringent quality control inspections are packaged and prepared for shipping.

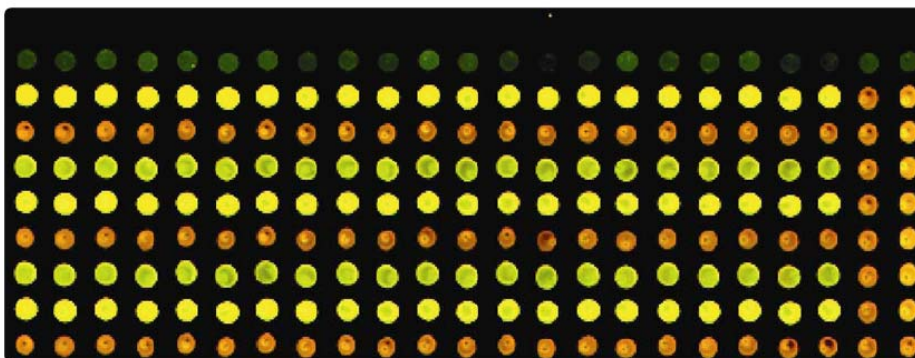
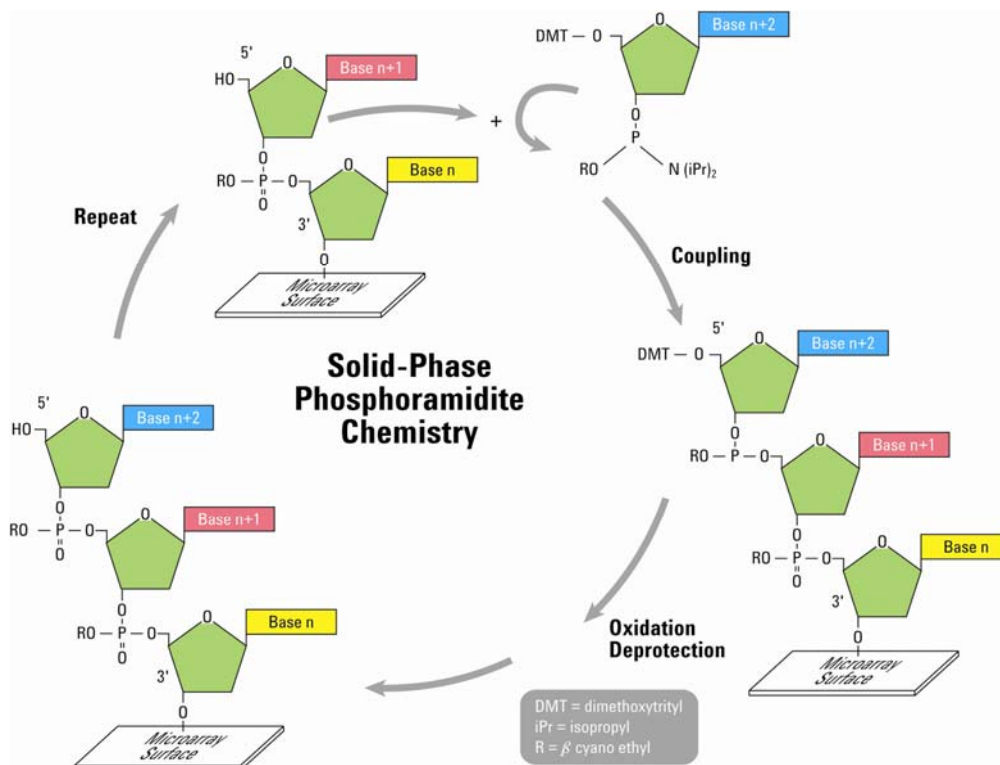


Figure 2. Superior feature printing. QC scan of an Agilent 60-mer oligo microarray showing a high degree of feature spatial uniformity and accurate grid placement.

Further Reading on Agilent Array Technology

Agilent's Microarray Platform: How High-Fidelity DNA Synthesis Maximizes the Dynamic Range of Gene Expression Measurements [http://www.chem.agilent.com/Library/applications/5989-9159Scanner%20app%20note%20\(2\).pdf](http://www.chem.agilent.com/Library/applications/5989-9159Scanner%20app%20note%20(2).pdf)

Link to animation video of SurePrint process: <http://www.chem.agilent.com/en-US/Search/Library/layouts/Agilent/PublicationSummary.aspx?whid=57450&iid=42>

Agilent SurePrint Technology Overview
<http://www.chem.agilent.com/Scripts/Generic.ASP?IPage=557&indcol=N&prodcol=Y>

Recommended Equipment to Start an Agilent Microarray Lab

Agilent arrays may be processed in an individual laboratory or through a core lab or service provider. The list below details the equipment necessary to process Agilent arrays.

Product Description	Part Number
Microarray Scanning	
DNA Microarray Scanner	G2565CA
Software	
Feature Extraction Software	
Sample Isolation and QC	
Agilent 2100 Bioanalyzer	
Agilent RNA 6000 Nano Reagents	5067-1512
Agilent RNA 6000 Nano Ladder	5067-1529
Microarray Hybridization	
DNA Microarray Hybridization Oven	G2545A
SureHyb Hybridization Chamber	G2534A
Hybridization Gaskets	
Hybridization Gasket Slide Kits 1 array/slide	
Hybridization Gasket Slide Kit (5)	G2534-60003
Hybridization Gasket Slide Kit (20)	G2534-60008
Hybridization Gasket Slide Kit (100)	G2534-60005
Hybridization Gasket Slide Kits 2 arrays/slide	
Hybridization Gasket Slide Kit (5)	G2534-60002
Hybridization Gasket Slide Kit (100)	G2534-60006
Hybridization Gasket Slide Kits 4 array/slide	
Hybridization Gasket Slide Kit (5)	G2534-60011
Hybridization Gasket Slide Kit (20)	G2534-60012
Hybridization Gasket Slide Kit (100)	G2534-60013
Hybridization Gasket Slide Kits 8 array/slide	
Hybridization Gasket Slide Kit (5)	G2534-60014
Hybridization Gasket Slide Kit (20)	G2534-60015
Hybridization Gasket Slide Kit (100)	G2534-60016

Certified Agilent Service Providers

Agilent offers a certification program for Microarray Service Labs that undergo additional on-site training and pass a rigorous set of quality assessments.

Service Providers-Americas

- Cogenics: www.cogenics.com
- Empire Genomics: www.empiregenomics.com
- ExonHit: www.splicearray.com
- GeneLogic: www.genelogic.com
- MOgene: www.mogene.com
- The Prostate Centre: www.mafpc.ca
- UHN Microarray Centre: www.microarrays.ca

Service Providers- Asia/Pacific

- Chemicals Evaluation and Research Institute, Japan: http://www.cerij.or.jp/ceri_en/index_e4.shtml
- DNA Chip Research Institute: <http://www.dna-chip.co.jp/>
- Genotypic: <http://www.genotypic.co.in/agilent.htm>
- Hokkaido System Science Co.: <http://www.hssnet.co.jp/index.html>
- Shanghai Biochip: <http://www.shbiochip.com/>
- Welgene: <http://www.welgene.com.tw/>

Service Providers – Europe/Middle East/Africa

- CXR Biosciences: <http://www.cxbiosciences.com/>
- Finnish DNA Microarray Centre: <http://microarrays.btk.fi/>
- IGR: <http://www.igr.fr/>
- imaGenes: <http://www.igr.fr/>
- IMGm Laboratories: http://www.imgm.de/rna_microarray.htm
- Miltenyi Biotec: <http://www.miltenyibiotec.com/>
- OGT: <http://www.ogt.co.uk/>
- University of Pavia