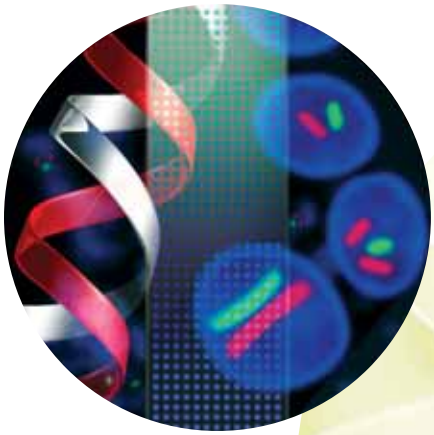
GENOMICS INFORMATICS PROTEOMICS METABOLOMICS

A T C T G A T C C T T C T G A A C G G A A C T A A T T T C A A
G A A T C T G A T C C T T G A A C T A C C T T C C A A G G T G

Agilent Human, Mouse and Rat Genome CGH Microarrays, 244A and 105A

Driving the Next Generation of High Performance

Genomic instability is a classic hallmark of cancer genetic disorders. The Agilent oligo array-based Comparative Genomic Hybridization (aCGH) platform lets you profile DNA copy number variations with superior resolution—all on a genome-wide scale. It delivers sensitivity and specificity that you can count on and has been carefully designed to meet the unique, challenging demands of the CGH application.



Agilent Human, Mouse and Rat Genome CGH Microarrays are now available in higher density formats. The 244A Microarray delivers five times more coverage for better scanning and precision in mapping chromosomal breakpoints, without compromising high performance and sensitivity. The 105A Microarray offers the flexibility of running two samples on one chip. No matter which format serves your needs, Agilent's next generation CGH microarrays allow you to improve your results, lower your costs, and follow where your research takes you, all with a single chip.

Features and benefits

Sensitivity and specificity

- Probe design and validation processes specifically optimized for aCGH
- Total genomic DNA analysis without amplification or complexity reduction
- Dynamic analytical range for challenging and complex, heterogeneous samples
- Low-input sample requirement

Flexibility

- 60-mer SurePrint *in situ* probe synthesis
- Multiplex arrays for lower cost per experiment
- Choice of genome-wide design and custom zoom-in content
- Scalable, high-throughput platform

Integrated informatics

- Intuitive GeneSpring CGH visualization and analysis interface
- Integration of aCGH and gene expression data

Quality support

- QC metrics for quality assessment

Optimized probe design and selection
Oligonucleotide probes were designed *in silico*, selected, and optimized for copy number detection using a multivariate approach. Overall system signal-to-noise ratios were significantly increased by reducing nonspecific noise. First, candidate probes representing unique genomic sequences were selected, scored, and filtered using bioinformatics prediction criteria for probe sensitivity, specificity, and responsiveness under appropriate conditions. Selection criteria were based on empirical testing of known genomic aberrations in comparative model systems, such as XX/XY hybridizations. The resulting candidate probe collection, spaced at ~200 bp intervals, was subjected to placement optimization for content coverage and relative spacing.

Probes on Agilent 244A and 105A microarrays span both coding and non-coding regions for comprehensive genome-wide representation (Table 1). There is an even distribution of multiple-probes across long and short transcripts. Additional emphasis is on microRNA representations using the Sanger miRNA registry (Figure 1), and promoter and sub-telomeric regions (data not shown).

Microarray Specifications

	Human Genome		Mouse Genome		Rat Genome	
Format	**244A**	**105A**	**244A**	**105A**	**244A**	**105A**
Microarrays per slide	1	2	1	2	1	2
Slides per kit	5	5	5	5	5	5
Slide format	1" x 3"	1" x 3"	1" x 3"	1" x 3"	1" x 3"	1" x 3"
Probe length	60-mer	60-mer	60-mer	60-mer	60-mer	60-mer
Feature size	65 µm	65 µm	65 µm	65 µm	65 µm	65 µm
Total features	243,504	105,072	243,504	105,072	243,504	105,072
Distinct biological features	236,381	99,026	235,402	98,797	235,974	97,973
Replicated biological features (in triplicate)	1,000	525	1,400	760	1,000	1,000
Agilent internal quality control features	5,045	4,626	5,099	4,712	5,492	5,041
Biological probes retained from earlier 44K format	97.9%	97.9%	98.9%	98.9%	N/A	N/A
Sequence source	UCSC hg17 (NCBI build 35)		UCSC mm7 (NCBI build 35)		UCSC rn4 (Baylor HGSC v3.4)	
Starting sample input	0.5 µg genomic DNA (direct labeling) or 0.05 µg genomic DNA (WGA amplification)					
Labeling type	Random priming using Cyanine 3 and Cyanine 5 nucleotides					
Overall assay time	3 days (1.5 days actual hands-on time)					
Storage condition	Room temperature (in the dark)					

Superior sensitivity and specificity

The Agilent aCGH platform delivers accurate copy number measurement as demonstrated in an XX and XY model system. The log ratio accuracy and detection sensitivity from analyzing 46, XY versus 46, XX genomic DNA samples on a 244A human microarray show excellent separability (99%) between autosomal and X-chromosome probes (Figure 2). In addition, the $\log_2$ ratio values derived from the maximal peaks for each probe tightly correlate to their expected theoretical values of 0 and -1, for one and two copies, respectively.

Detection of microdeletions and microamplifications enables the identification of novel oncogenes, tumor suppressor genes, and biomarkers associated with cancer and other genetic disorders. Agilent aCGH analysis—with both 244K and 105K formats—of the well-characterized HT29 human colon carcinoma cell line detected known large amplifications and deletions as well as a focal deletion involving a transcription factor and tumor suppressor that was not reported in any previous BAC array-based analyses (Figure 3).

Enhanced precision and novel detection

Most cancers result from the combination of acquired genomic instabilities within cells throughout their lifecycle and the subsequent clonal expansion of these genetically altered cells with cumulative genetic errors. These genomic variations include gains and losses varying in size from entire chromosomes, to chromosomal regions, to specific, focal gene variations. The enhanced detection resolution of Agilent 244A and 105A microarrays delivers exquisitely fine precision. The platform readily maps chromosome breakpoints (Figure 4) and identifies microvariations (Figure 5) that no other technology platform can detect.

Agilent Genomic DNA Labeling Kit PLUS

The latest addition to the aCGH reagents line is the Agilent Genomic DNA Labeling Kit PLUS. It was specially developed, validated, and optimized for the Agilent oligo aCGH workflow. Using a simple, one-tube reaction, it quickly generates Cyanine-labeled genomic DNA targets from as little as 0.5 µg total genomic DNA. The PLUS Kit contains all required components—including Cyanine 3- and 5-dUTP dyes, to complete a total of 50 labeling reactions.

Format		Human Genome 244A	Human Genome 105A	Mouse Genome 244A	Mouse Genome 105A	Rat Genome 244A	Rat Genome 105A
Intragenic probes		70.0%	71.0%	68.4%	69.6%	48.8%	50.5%
Intergenic probes		30.0%	29.0%	31.6%	30.4%	51.2%	49.5%
RefGenes represented		97.9%	97.9%	98.3%	98.3%	99.6%	99.6%
Median probe spacing	Overall	8.9 KB	21.7 KB	7.8 KB	19.3 KB	8.0KB	19.1KB
	Intragenic	7.4 KB	18.9 KB	6.2 KB	15.7 KB	4.7KB	11.4KB
	Intergenic	16.5 KB	50.6 KB	15.2 KB	36.8 KB	14.9KB	37.1KB
Average probe spacing*		6.4 KB	15.0 KB	6.4 KB	15.0 KB	6.0KB	14.5KB

*Calculated by dividing total repeat-masked genome size by total microarray features.

Table 1. Probe Coverage Specifications

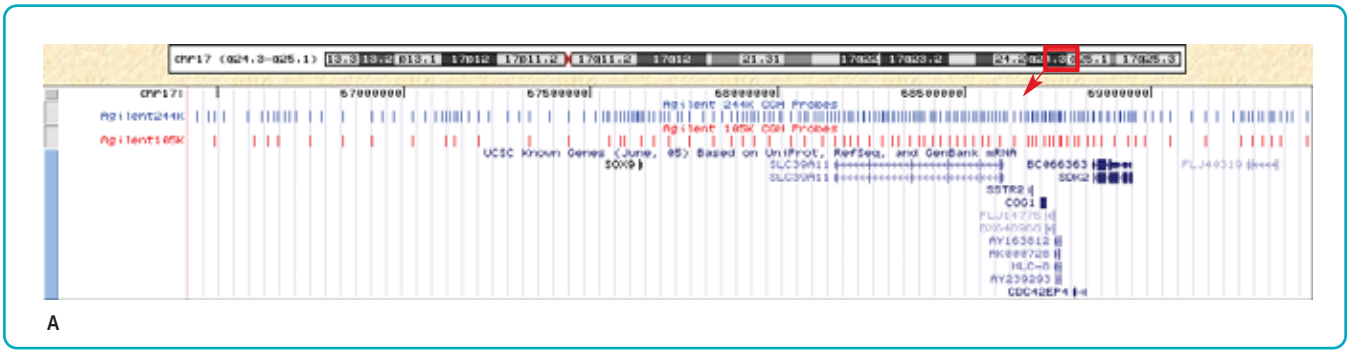


Figure 1. Typical probe coverage characteristics on Agilent 244A and 105A CGH microarrays as illustrated using UCSC hg18 Human Genome Browser, Feb 2006 (NCBI build 36) of human chromosome 17. At the top of each view, each short vertical bar represents a single probe on Agilent Human Genome CGH 244A (blue) or 105A (red). Annotations below the probes indicate the location mapping of UCSC known genes (blue or black) and Sanger microRNAs (red). **(A)** A 3.0 MB microarray window of 17q24-25 shows comprehensive coverage over coding and non-coding regions. **(B)** A 3.0 MB window of 17q11-12 depicts evenly distributed multiple-probe coverage across long (ACCN1) as well as short (NLE1) transcripts. **(C)** A 1.0 MB region of 17q11.2 illustrates microRNA representations (hsa-mir-193a, hsa-mir-365-2).

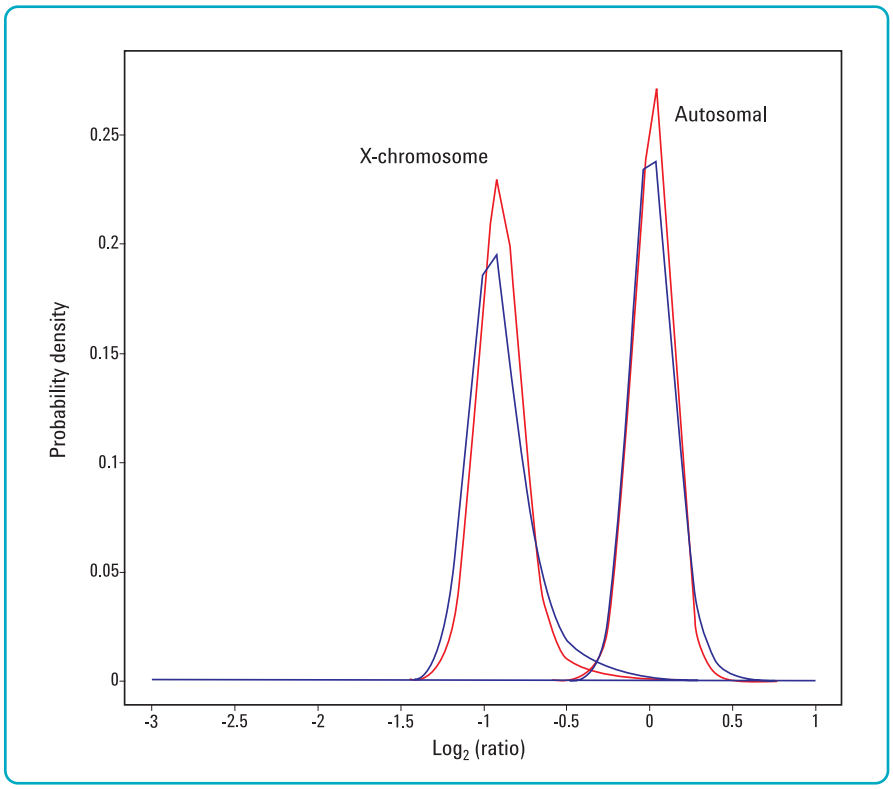


Figure 2. A representative separation histogram showing distribution of $\log_2$ ratio values of X chromosome probes (left peaks) versus autosomal probes (right peaks) in a single dye-flip pair of XY/XX hybridizations on the Agilent Human Genome CGH Microarray 244A (P/N G4411B).

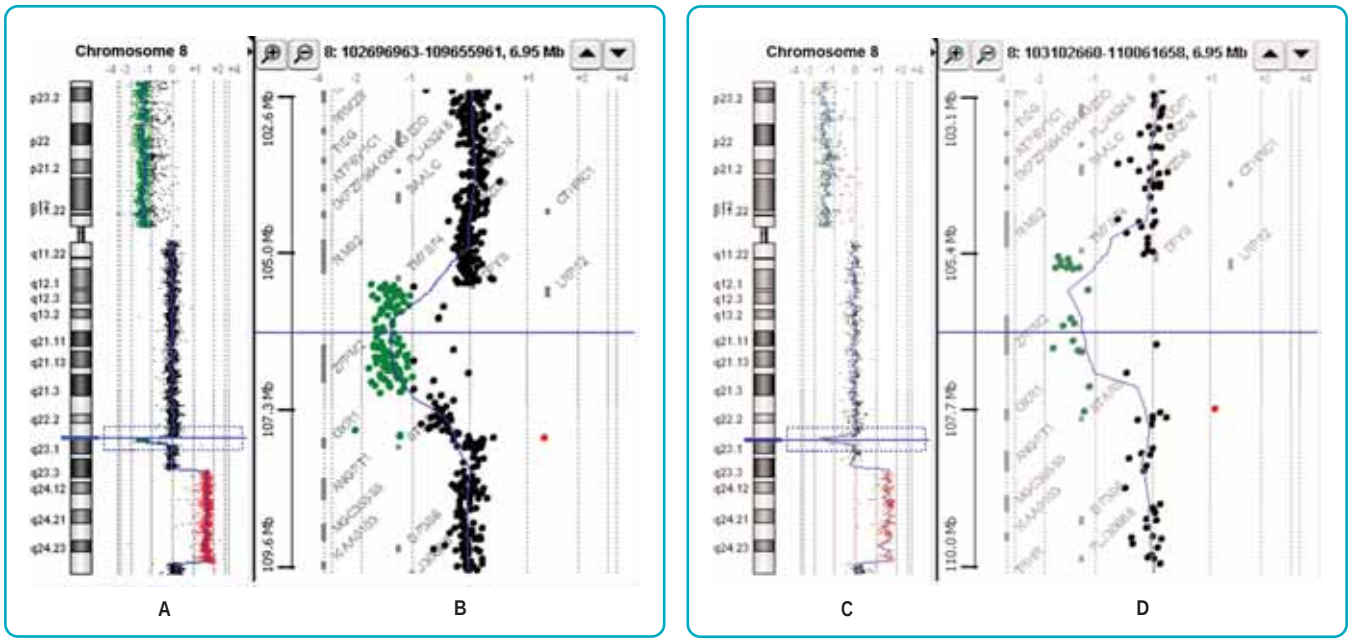
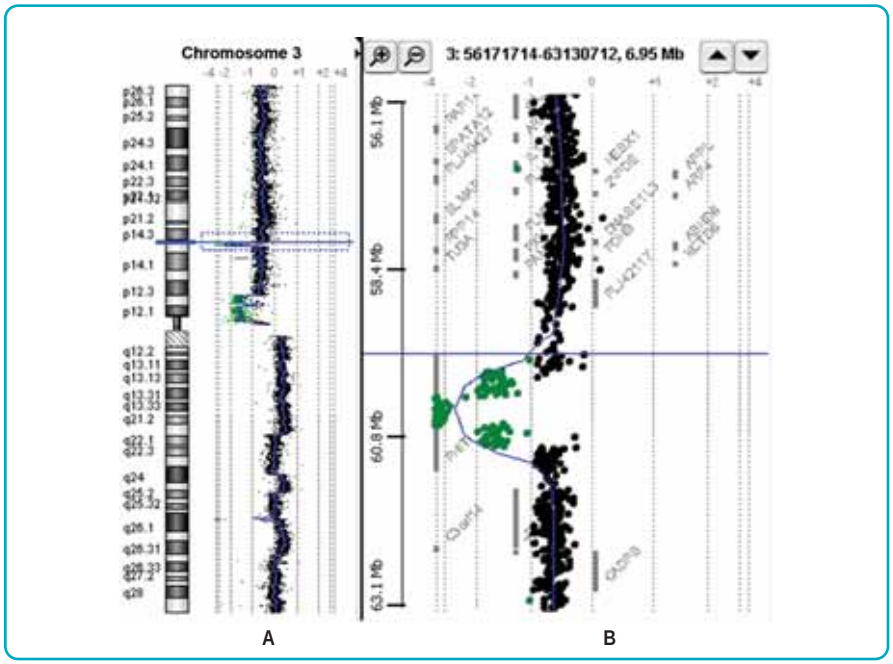


Figure 3. CGH Analytics views of Agilent 244K and 44K analyses of chromosome 8 in the human colon carcinoma cell line HT29. **(A)** Scatter plot (chromosome view) produced from an Agilent Human Genome CGH Microarray 244A (P/N G4411B) analysis reveals a substantial deletion across the p arm (horizontal shift to left of zero line), amplification along the q arm in the q23.3-24.23 region (horizontal shift to right of zero line), and a focal deletion in q23.1 (horizontal shift to left of zero line, outlined by dotted blue box). **(B)** Zoomed-in gene view of panel A which focuses on a 7 MB window within q23.1 containing the focal deletion. It distinctly shows a ~1.5 MB deletion involving the zinc finger protein ZFPM2 and tumor suppressor LRP12 genes. **(C and D)** Parallel scatter plots (chromosome and gene views, respectively) from an Agilent Human Genome CGH Microarray 44B (P/N G4410B) analysis of the same sample as in panels A and B.



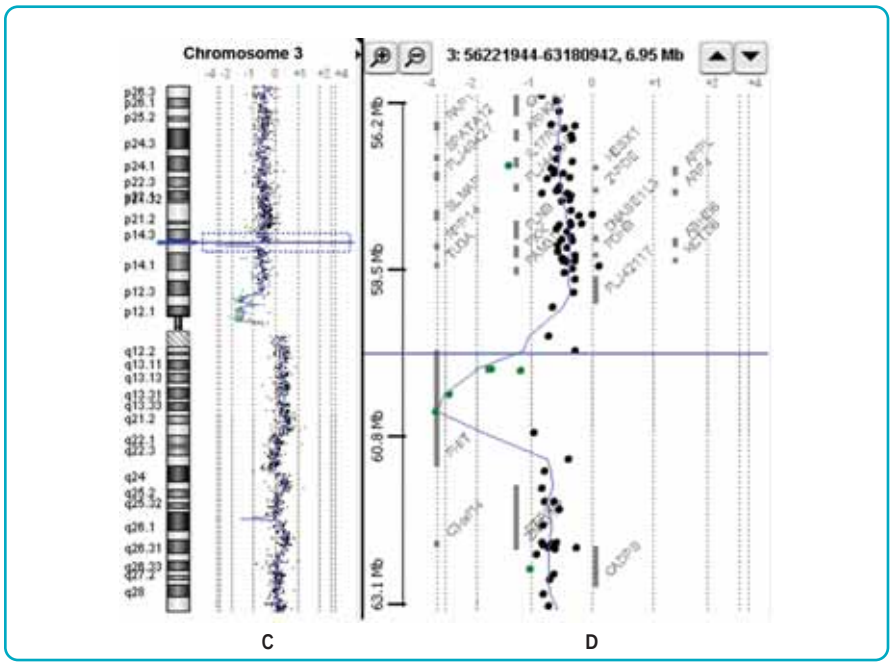


Figure 4. CGH Analytics views of Agilent 244K and 44K analyses of chromosome 3 in the human colon carcinoma cell line HT29. **(A)** Scatter plot (chromosome view) produced from an Agilent Human Genome CGH Microarray 244A (P/N G4411B) analysis reveals multiple p arm deletions in regions such as p12.1-12.3 (horizontal shift to left of zero line) and a focal deletion in p14.2 (horizontal shift to left of zero line, outlined by dotted blue box). **(B)** Zoomed-in gene view of panel A which focuses on a 7 MB window within p14.2 containing the focal deletion. It distinctly shows two different deletion patterns both within the single tumor suppressor FHIT gene. **(C and D)** Parallel scatter plots (chromosome and gene views, respectively) from an Agilent Human Genome CGH 44B Microarray (P/N G4410B) analysis of the same sample as in panels A and B.

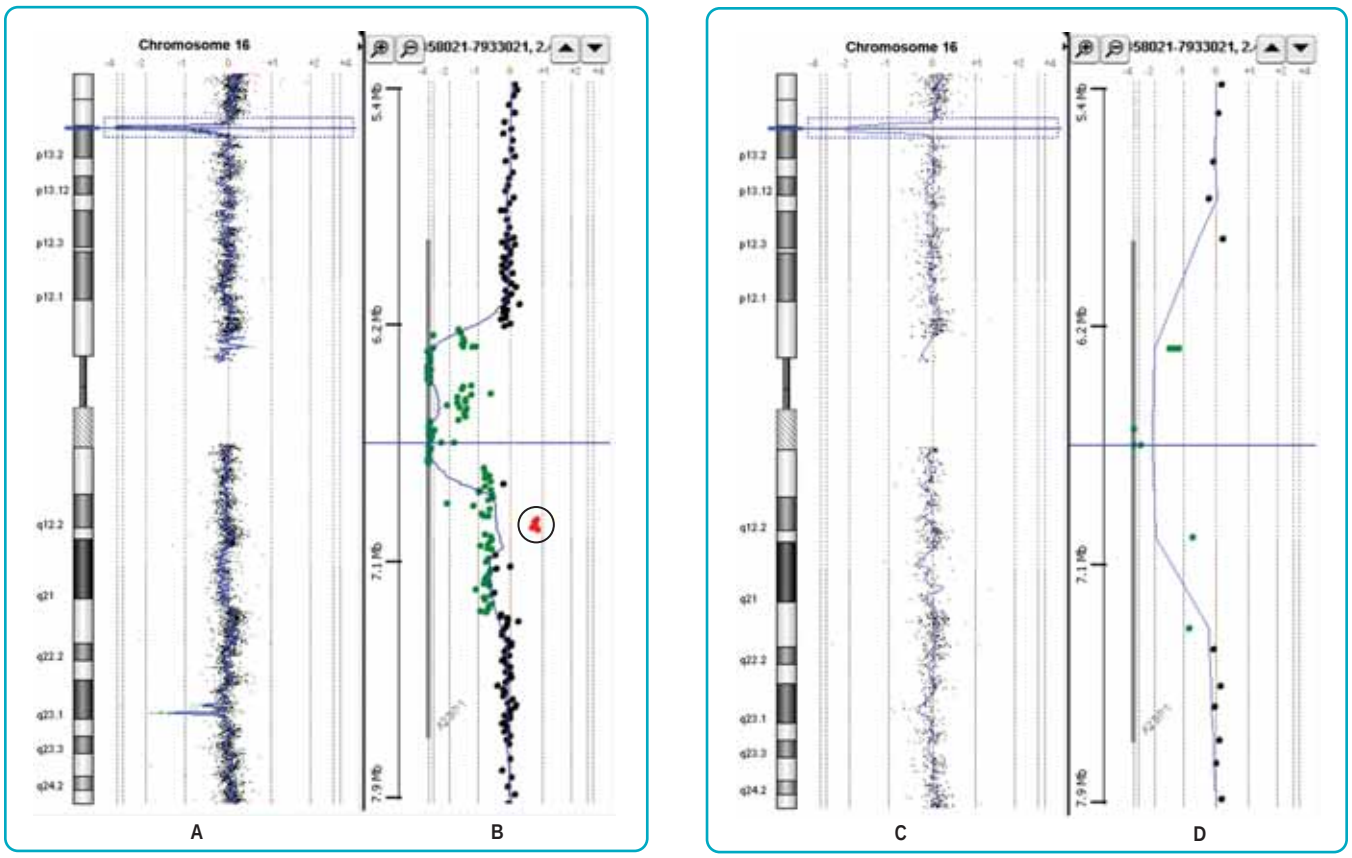


Figure 5. CGH Analytics views of Agilent 244K and 44K analyses of chromosome 16 in the human colon carcinoma HT29 cell line. **(A)** Scatter plot (chromosome view) produced from an Agilent Human Genome CGH Microarray 244A (P/N G4411B) analysis reveals a focal deletion in p13.2 (horizontal shift to left of 0, outlined by dotted blue box). **(B)** Zoomed-in gene view of panel A which focuses on a 2.5 MB window within p13.2 containing a focal deletion. It readily detects multiple deletion patterns within the ataxin-2 binding protein A2BP1 gene (green dots) and a <50 KB microamplification (red dots within circle). This microamplification was verified by multiple consecutive probes. **(C and D)** Parallel scatter plots (chromosome and gene views, respectively) from an Agilent Human Genome CGH Microarray 44B (P/N G4410B) analysis of the same sample as in panels A and B. In this particular example, while the 44B format detected the A2BP1 deletion, it detected neither the variable deletion patterns nor the microamplification.

Ordering Information

Description	Part Number
Agilent CGH Microarrays	
Human Genome CGH Microarray Kit 244A	G4411B
Human Genome CGH Microarray Kit 105A	G4412A
Mouse Genome CGH Microarray Kit 244A	G4415A
Mouse Genome CGH Microarray Kit 105A	G4416A
Rat Genome CGH Microarray Kit 244A	G4435A
Rat Genome CGH Microarray Kit 105A	G4436A
Required Agilent CGH Microarray Processing Components	
Agilent Genomic DNA Labeling Kit PLUS (50)	5188-5309
Agilent Oligo aCGH Hybridization Kit (25) or (100)	5188-5220 or 5188-5380
Agilent Oligo aCGH Wash Buffer 1 and 2 Set	5188-5226
Hybridization Chamber, stainless	G2534A
Hybridization Chamber Gasket Slides, 5-pack+	G2534-60003 or G2534-60002
Hybridization Oven	G2545A
Hybridization Oven Rotator Rack	G2530-60029
Agilent Microarray Scanner Bundle (including Feature Extraction Software)*	G2565BA
CGH Analytics (Workstation or Concurrent)	G4172AA, G4173AA, G4174AA, G4176AA, G4177AA, or G4178AA
Optional Agilent CGH Microarray Processing Components	
Agilent Oligo aCGH Wash Buffer 1, 4L	5188-5221
Agilent Oligo aCGH Wash Buffer 2, 4L	5188-5222
Stabilization and Drying Solution, 500 ML	5185-5979

* Feature Extractions software can be purchased separately.

+ Alternative packaging size is available.

Kit Contents

- Five or ten microarrays printed on five 1" x 3" glass slides. Microarrays are shipped with foil seal. After breaking the foil, store microarrays at room temperature, in the dark, under a vacuum desiccator or in an N_2 purge box. Do not expose microarrays to open air during storage.
- CD-ROM containing microarray layout, annotation information, and recommended processing protocols

"Oligonucleotide Array-Based CGH for Genomic DNA Analysis" (Publication No. G4410-90010) is the system guide containing Agilent-recommended aCGH procedures for sample preparation, microarray processing, and data extraction. It is available for download at **www.agilent.com/chem/goCGH**.

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Printed in the U.S.A. March 19, 2007

5989-5499EN

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