Agilent SurePrint G3 Human Catalog CGH Microarrays

Product Note

"The new Agilent 1M CGH array combines the excellent probe design and performance characteristics of the Agilent aCGH platforms with a million feature microarray resulting in extremely clean data and a functional resolution of 4kb that far exceeds any platform we have tested to date."

Jonathan Keats, PhD
Research Fellow
Mayo Clinic Arizona

"We have recently tested the new Agilent one million probe human CGH arrays using genomic DNA isolated from both normal and tumor tissue specimens. The increase in probe density has allowed us to identify precise margins of chromosome aberrations that define specific breakpoints, regions of minimal deletion, and tightly focused amplicons. Agilent's one million arrays provide the benefit of more genomic content with the same acquisition of high quality data that we have experienced with earlier formats."

Angela S. Baker, Ph.D.,
Staff Biologist
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Translational Genomics
Research Institute (TGen)

The Agilent SurePrint G3 Human Catalog 1x1M, 2x400K, 4x180K, and 8x60K CGH Microarrays contain four times more empirically validated probes for detecting copy number variation than the corresponding legacy 1x244K, 2x105K, 4x44K, and 8x15K SurePrint HD arrays. The SurePrint G3 CGH microarrays utilize the industry's highest fidelity long-mer probes resulting in the most accurate detection of copy number measurements. The Agilent CGH platform consists of flexible multi-pack formats for both catalog and custom arrays and enables copy number analysis of most sample types, including FFPE, using the robust DNA Analytics analysis tool. The quality, flexibility, and complete workflow combine to make Agilent CGH microarrays the highest performing copy number solution.

Introduction

Agilent has consistently provided powerful catalog and custom arrays for the study of copy number changes using array comparative genomic hybridization (aCGH). The Agilent CGH offering now includes higher density by the introduction of the 1x1M, 2x400K, 4x180K, and 8x60K formats, also known as SurePrint G3 Microarrays. The SurePrint G3 Microarrays continue Agilent's record for high sensitivity and high specificity while increasing resolution to map copy number aberration breakpoints, identify smaller gains and losses, and provide more comprehensive whole genome coverage. These newer formats are still printed on 1 in. x 3 in. glass slides and are compatible with the standard hybridization gaskets and chambers. SurePrint G3 CGH Microarrays are scanned at 3 microns using the Agilent SureScan Microarray Scanner (G4900DA). Feature Extraction (part of Agilent CytoGenomics or Agilent Genomic Workbench) then seamlessly translates the resulting image into log ratios and uses advanced statistical analysis to associate these with correlative QC metrics. Results can be further analyzed and displayed for biological interpretation in Agilent CytoGenomics or DNA Analytics (part of Agilent Genomic Workbench).

Agilent Technologies

We developed a database of 28 million distinct oligonucleotide probes suitable for aCGH at median spacing of less than 100 bp within the non-repeat masked regions of the genome. Probes were selected using an empirical model that applies scores for homology, thermodynamics, secondary structure and sequence complexity. The catalog 1x1M, 2x400K, 4x180K, and 8x60K arrays were designed from this probe database. The entire database of over 28 million probes, including all catalog probes, are available in eArray, a free web tool provided by Agilent for the design of custom microarrays. Genomic DNA from blood, cells, tissues, and FFPE can be utilized with these arrays to answer your biological questions.

Together with Agilent CytoGenomics or Agilent Genomic Workbench, Agilent SurePrint G3 Human CGH Microarrays allow you to interrogate more of the genome at a lower cost, enabling you to take your research farther.

Microarray Specifications

Table 1 lists the microarray specifications. These new catalog designs contain new probes in both the coding and non-coding regions of the genome, pseudoautosomal regions, and sub-telomeric regions. In addition, the vast majority of the probes from the 244K and 44K legacy arrays are also present for backwards compatibility. The increased density results in 2.1-kb median probe spacing on the SurePrint G3 CGH 1x1M Microarray, allowing for the detection of smaller, more focal amplifications and deletions (see Biological Results section below).

Microarray Performance

The Agilent SurePrint G3 Human Catalog CGH Microarrays provide accurate copy number measurements, as illustrated in Figure 1. By using a Male/Female model system, the log ratio accuracy and detection sensitivity can be determined. Figure 1 shows the separation between the autosomal and X chromosome probe $\log_2$ ratios (Area under ROC curve of 98%). In addition, the $\log_2$ ratio distributions are centered on the

Table 1. Microarray specifications

		1x1M	2x400K	4x180K	8x60K
Design ID		021529	021850	022060	021924
Total features		974,016	420,288	180,880	62,976
Control grid feature count		6,685	5,126	6,539	3,886
Distinct biological features		963,029	411,056	170,334	55,077
Replicated probes (5x)		1,000	1,000	1,000	1,000
Additional neg. controls		302	106	7	13
Retained 244K probes		225,499 (95.4%)	225,476 (95.4%)	157,698 (66.7%)	49,413 (20.9%)
Retained 105K probes		93,095 (94.0%)	92,891 (93.8%)	88,244 (89.1%)	47,648 (48.1%)
Retained 44K probes		38,621 (90.9%)	38,602 (90.8%)	38,621 (90.9%)	38,621 (90.9%)
Genome build		hg18	hg18	hg18	hg18
Unique probes		961,035 (99.8%)	410,184 (99.8%)	169,991 (99.8%)	54,969 (99.8%)
Homology filtered probes		4,642 (0.48%)	1,634 (0.40%)	511 (0.28%)	149 (0.27%)
Pseudoautosomal probes		1,994	872	343	108
Exonic probes		51,297 (5.3%)	35,945 (8.7%)	24,011 (14.1%)	14,259 (25.9%)
Intragenic probes		514,337 (53.4%)	218,058 (53.0%)	93,675 (55.0%)	36,995 (67.2%)
Intergenic probes		448,692(46.6%)	192,998 (47.0%)	76,659 (45.0%)	18,082 (32.8%)
Median probe spacing	**Intragenic**	1,814	4,606	11,190	33,307
	Intergenic	2,752	6,876	17,655	78,946
	CNV	2,092	5,246	12,690	26,688
	Overall	2,106	5,315	13,066	41,448
Average probe spacing	**Overall**	3,118	7,304	17,627	54,455
RefSeq coverage (18,698 genes)	**At least 1 probe**	17,551 (93.9%)	17,234 (92.1%)	16,605 (88.8%)	15,553 (83.2%)
	≥3 probes	15,180 (81.2%)	12,530 (67.0%)	8,673 (46.4%)	4,580 (24.5%)
Cancer gene coverage (362 genes)	**At least 1 probe**	358 (98.9%)	358 (98.9%)	355 (98.0%)	351 (97.0%)
	≥3 probes	344 (95.0%)	316 (87.3%)	280 (77.3%)	226 (62.4%)

expected values of 0 for the autosomal probes (corresponding to two copies) and –1 for the X chromosome probes (corresponding to one copy). The pseudoautosomal probes are plotted separately so as not to result in false positive values on the X chromosome, since they are represented on both chromosomes X and Y and therefore are expected to have a $\log_2$ ratio of 0.

The QC metrics from DNA Analytics (part Agilent Genomic Workbench) are listed in Table 2. These results were generated using the *Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis, Enzymatic Labeling for Blood, Cells or Tissue with a High Throughput option version 6 User Manual*. This assay is similar to the legacy assay recommended with the SurePrint HD arrays, with improvements discussed below. The new SurePrint G3 microarrays show a similar performance as the legacy SurePrint HD arrays across a variety of sample types.

Biological Results

Agilent SurePrint G3 Human Catalog CGH Microarrays can be used for many different types of applications such as cancer biology and cytogenetics. The ability to accurately detect and quantify microdeletions and microamplifications is key to understanding the biology in these applications. Using the SurePrint G3 Catalog CGH 1x1M Microarray on a Burkitt lymphoma tumor sample, a small deletion on chromosome 2 predicted by a single probe on the 244K microarray was confirmed by six probes clearly delineating the breakpoints (Figure 2A). The complex structure of multiple aberrations within the WWOX gene region in the Burkitt lymphoma sample was clarified on the SurePrint G3 Catalog 1x1M Microarray due to the higher density of probe coverage (Figure 2B). The improvements in density did not compromise performance. As can be seen in the retinoblastoma sample, the response to copy number was maintained in the SurePrint G3 Catalog CGH 1x1M Microarray, such that the $\log_2$ ratio for a hemizygous deletion on chromosome 13 was presented as expected at –1 (Figure 2C).

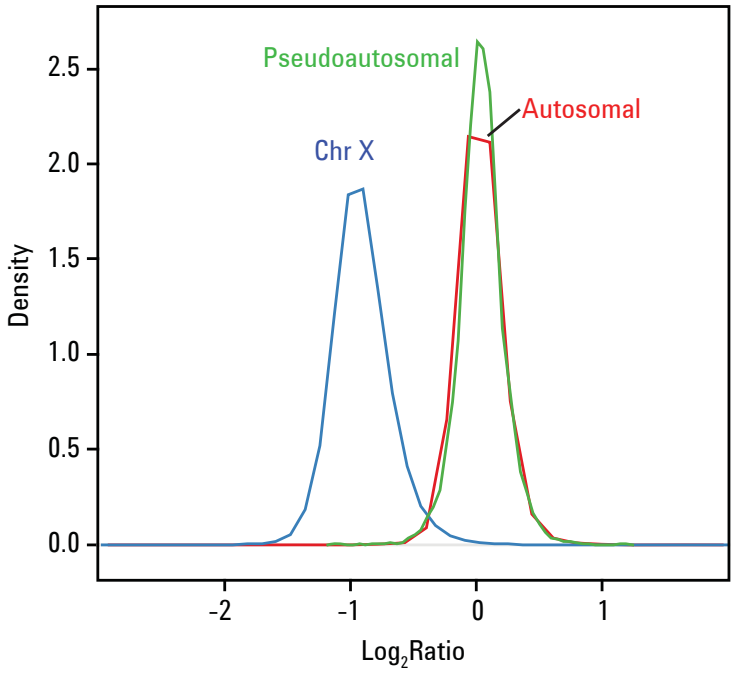

Figure 1. A representative separation histogram showing the distribution of $\log_2$ ratio values for X chromosome probes (blue), pseudoautosomal probes (green) and autosomal probes (red) from an XY/XX hybridization on the Agilent SurePrint G3 Catalog CGH 1x1M CGH Microarray (P/N G4447A).

1x1M	Male-Female (n=4)	All samples (n=18)
DLRSpread	0.17±0.01	0.19±0.03
SignalToNoiseGreen	58.1±10.4	59.0±13.3
SignalToNoiseRed	55.7±3.9	53.8±8.5
SignalIntensityGreen	120.7±7.9	142.3±22.8
SignalIntensityRed	175.8±9.1	192.1±33.6
BGNoiseGreen	2.2±0.6	2.6±1.0
BGNoiseRed	3.2±0.4	3.6±0.8
ReproducibilityGreen	9.8±0.7	9.8±1.0
ReproducibilityRed	10.3±0.9	10.7±1.0
AreaUnderROC	0.98±0.003	na
MedianDiff	0.95±0.014	na

Table 2. QC results for Agilent SurePrint G3 Catalog CGH 1x1M Microarrays. Average scores are provided along with standard deviations across 4 normal female samples hybridized against normal male samples and a range of tumor and cytogenetic samples labeled with the Genomic DNA Enzymatic Labeling kit (n=18).

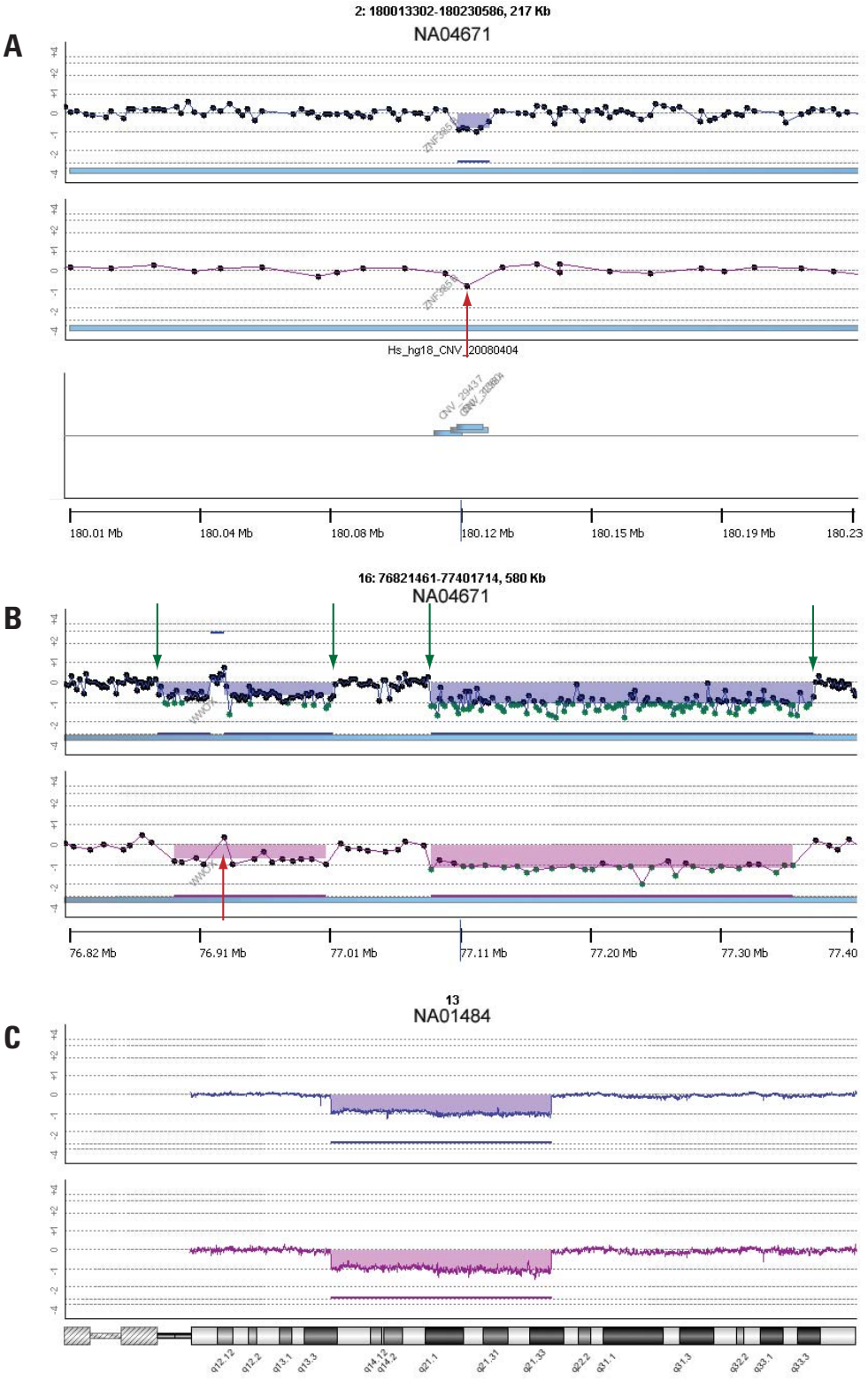

Figure 2. DNA Analytics views of Agilent SurePrint G3 CGH 1x1M and SurePrint HD 244K Microarrays.

(**A**) Confirmation of single probe call (red arrow) on 244K microarray with a six probe call (~10 Kb) on the SurePrint G3 CGH 1x1M Microarray. Deletion overlaps with a known Copy Number Variant (CNV) region from Toronto Database of Genomic Variants. (**B**) Refinement of breakpoints (green arrows) and structure (red arrow) in WWOX gene in a Burkitt Lymphoma sample. (**C**) A Retinoblastoma sample (NA01484) showing that the response to copy number was maintained in the SurePrint G3 Catalog CGH 1x1M Microarray, 0.2 Mb moving average. DNA Analytics settings: ADM-2, threshold 5, filter: 2 probes, 0.25 $\log_2$ ratio.

Data from samples containing known cytogenetic aberrations are displayed in Figure 3. The expected gains and losses were consistently and confidently identified based on published information. Another way to view the results is within the UCSC Genome Browser. Figure 4 (see page 6) shows a Prader-Willi Syndrome sample containing the deletion of an exon in the SNRPN gene on Chromosome 15 as indicated by the black bar. This focal deletion was undetectable on the 44K array due to lower probe coverage (data not shown). These data illustrate that the SurePrint G3 CGH 1x1M Microarray provides the combined advantage of increased coverage and higher density with consistent and excellent performance.

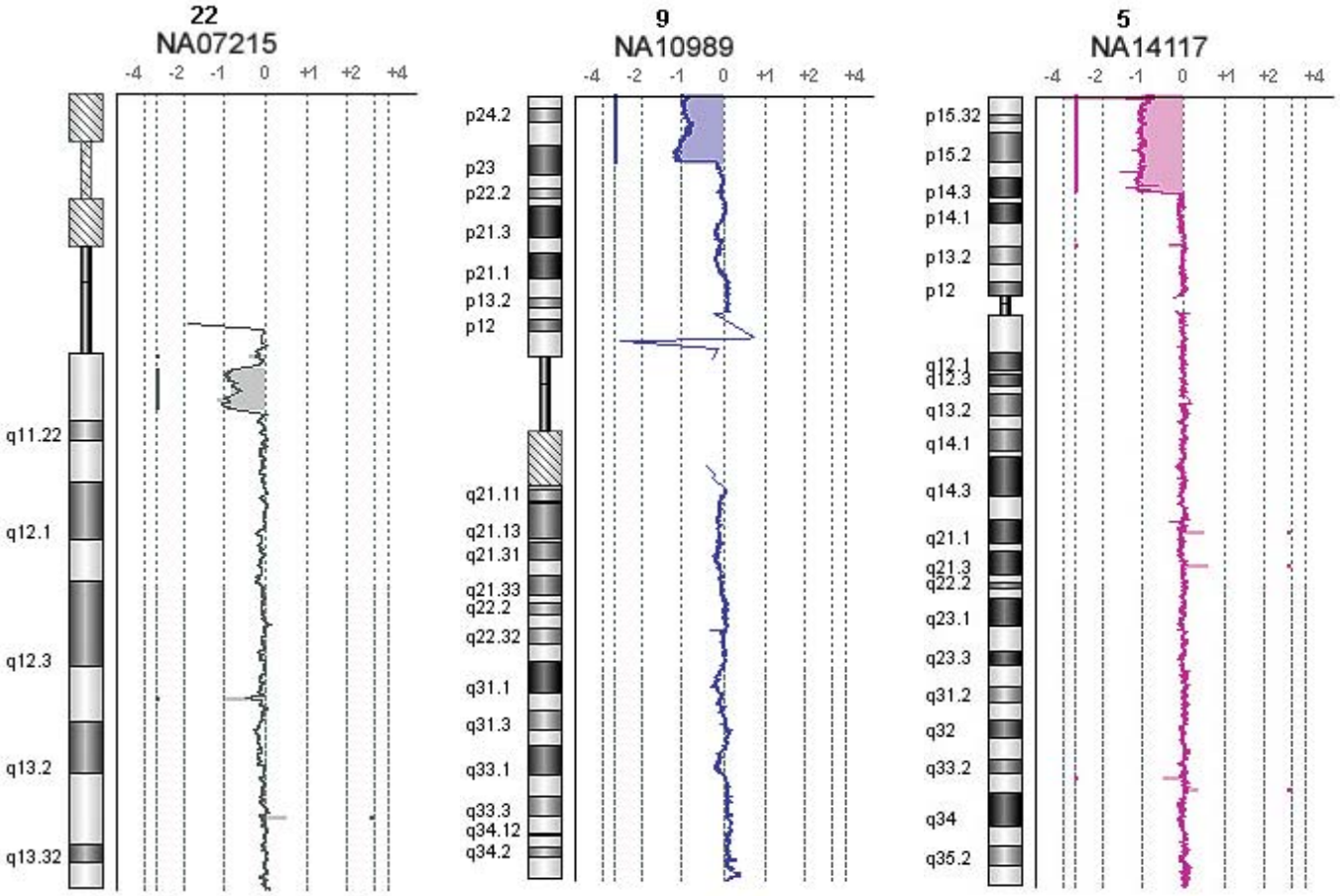

Figure 3. DNA Analytics views of Agilent SurePrint G3 CGH 1x1M Microarrays across a spectrum of cytogenetics samples. From left to right: NA07215, NA10989, and NA14117. All aberrations are consistently and confidently identified based on published information. DNA Analytics settings: ADM-2, threshold 5, filter: 2 probes, 0.25 $\log_2$ ratio, 0.2 Mb moving average.

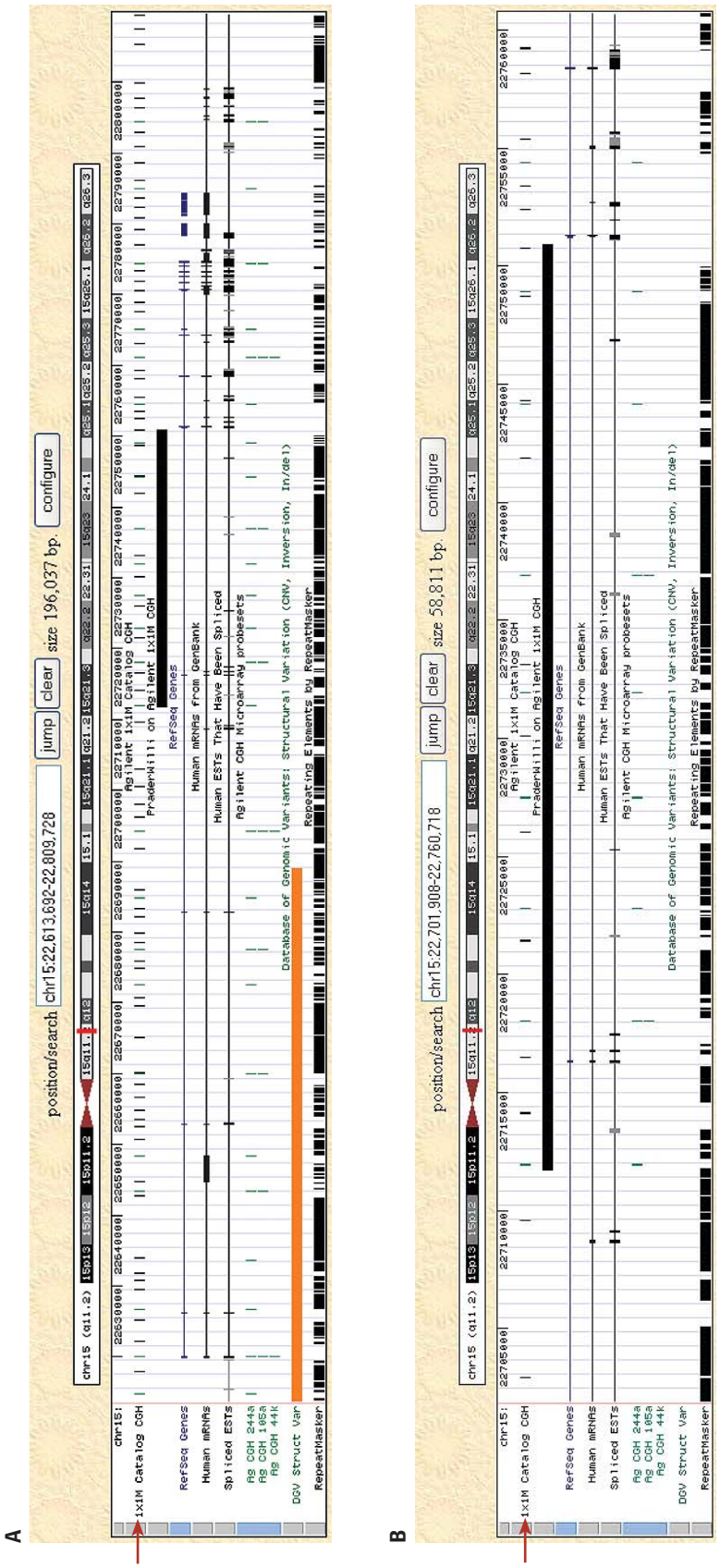

Figure 4. UCSC Genome Browser views of NA13555. Aberrations were loaded into UCSC browser in BED file format. Agilent 1x1M catalog CGH probes are displayed above aberration (see red arrow). Legacy Agilent catalog probes are displayed in green. (**A**) ~200-kb window show SNRPN gene. (**B**) Zoomed-in view of deleted region (~60 kb).

Improved Assay Throughput

Agilent offers two genomic DNA labeling options: enzymatic and ULS. The enzymatic option uses a random-primed Klenow fill-in based incorporation of Cy-3 or Cy-5 labeled dUTPs. The ULS or Universal Linkage System utilizes a non-enzymatic method to directly label genomic DNA with fluorescent dyes. Table 3 compares the two methods and researchers can choose between the two based on their experimental needs.

Both User Manuals (*Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis, Enzymatic Labeling for Blood, Cells or Tissue with a High Throughput option version 6* or higher and *Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis, ULS Labeling for Blood, Cells, Tissue, or FFPE with a High Throughput option version 3* or higher) contain updates for processing the SurePrint G3 CGH Microarrays. In addition, both methods support processing samples in 96-well plates. For the enzymatic method, the SureTag DNA Labeling Kit (5190-3400) is available, and Agilent also recommends the GE Healthcare 96-well plates (AutoScreen-96A part number 25-9005-98) for high-throughput purification of enzymatically labeled gDNA. For the ULS method, there are two kits available: Genomic DNA High-Throughput ULS Labeling kit (5190-0450) and Genomic DNA 96-well Purification Module (5190-0451). The Genomic DNA High-Throughput ULS Labeling kit contains higher volumes to allow for processing of more samples using the ULS methodology. The Genomic DNA 96-well Purification Module contains a 96-well purification plate, wash plate, and collection plate.

Conclusion

The SurePrint G3 CGH Microarrays combine the advantages of increased coverage and higher density with consistent and excellent performance. They have been shown to provide performance similar to the legacy SurePrint HD microarrays across a wide range of applications, including cancer biology and cytogenetics, where accurate detection and quantification of microdeletions and microamplifications is of key importance. The SurePrint G3 CGH Microarrays continue Agilent's record for high sensitivity and high specificity, while increasing resolution to map copy number aberration breakpoints, identify smaller gains and losses, and provide more comprehensive whole genome coverage. The quality, flexibility, and complete workflow combine to make Agilent's CGH Microarrays the highest performing copy number solution.

Table 3. Comparison of Enzymatic and ULS Labeling Methodologies*

Feature	Enzymatic Kit	ULS Kit
Sample compatibility	Cells, Frozen Tissue, Blood	Cells, Frozen Tissue, Degraded DNA, Blood, and FFPE
Sample requirement for 1 packs	500 ng	1500 ng**
Single sample processing time	4.5 hr	1 hr
Cost	Same cost for 1-, 2-, and 4-pack array formats; cost halved for 8-packs	Cost per sample less than enzymatic pricing; reduced cost with multipacks

* For more detailed information and guidance on the labeling kits, please see the User Manuals.
** 2000 ng input gDNA required for FFPE samples

Agilent SurePrint G3 CGH Microarrays

Description	Number of Arrays/Slide	Number of Slides/Kit	Part Number
SurePrint G3 Human CGH Microarray 1x1M	1	5	G4447A
SurePrint G3 Human CGH Microarray 2x400K	2	5	G4448A
SurePrint G3 Human CGH Microarray 4x180K	4	3	G4449A
SurePrint G3 Human CGH Microarray 8x60K	8	3	G4450A
SurePrint G3 Custom CGH Microarray 1x1M	1	1	G4123A
SurePrint G3 Custom CGH Microarray 2x400K	2	1	G4124A
SurePrint G3 Custom CGH Microarray 4x180K	4	1	G4125A
SurePrint G3 Custom CGH Microarray 8x60K	8	1	G4126A

NOTE: 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.

Required Agilent CGH Processing Components

Description	Part Number
SureTag Complete DNA Labeling Kit	5190-4240
Human Cot-1 DNA	5190-3393
Genomic DNA ULS Labeling Kit	5190-0419
Genomic DNA Purification Module (for ULS labeling)	5190-0418
Genomic DNA High-Throughput ULS Labeling Kit	5190-0450
Genomic DNA 96-well Purification Module (for ULS labeling)	5190-0451
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	Part number varies by array format and quantity
Hybridization Oven	G2545A
Hybridization Oven Rotator Rack	G2530-60029
SureScan Microarray Scanner	G4900DA
Agilent CytoGenomics	G1662AA–G1667AA
DNA Analytics	G4172AA

* Feature Extraction software can be purchased separately.

Optional CGH Processing Components

Description	Part Number
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
AutoScreen-96A Well plates	GE Healthcare p/n 25-9005-98

Recommended aCGH Procedures

Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis, Enzymatic Labeling for Blood, Cells or Tissue with a High Throughput Option, version 6 or higher (Publication No. G4410-90010) and *Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis, ULS Labeling for Blood, Cells, Tissue, or FFPE with a High Throughput Option, version 3* or higher (Publication No. G4410-90020) are system guides containing Agilent-recommended aCGH procedures for sample preparation, microarray processing, and data extraction. They are available for download at **www.agilent.com/genomics/microarrays**.

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