

# Agilent CytoGenomics Software

## *A Complete Solution for Cytogenetic Research Data Analysis*

### Technical Overview

**Streamlines the cytogenetic research workflow for finding CNCs, LOH, and UPD**

**Enables manual sample analysis with an in-house and external databases**

**Offers full automation of the analysis process**

**Provides easy-to-generate customizable analysis reports**

Cytogenetic research labs are increasingly turning to array CGH to improve detection of copy number changes and increase sample throughput. Alongside Agilent's gold standard CGH+SNP and CGH microarrays, Agilent CytoGenomics software completes our array-based cytogenetic research solution. Agilent CytoGenomics provides a statistically and visually powerful tool to streamline the day-to-day cytogenetic sample analysis research workflow. It offers full support for efficient analysis of cytogenetic samples and easy report generation, based on information from both external databases and an internal database. Key automation features enable a seamless flow through the experimental process, starting with full automation of data upload and analysis and ending with customized report generation. With Agilent CytoGenomics, cytogenetic research labs can analyze a normal, constitutional sample in less than five minutes. This increases efficiency and throughput while enabling cytogenetic researchers to better detect copy number and copy-neutral aberrations across the genome.

### Data Analysis Tool for the Cytogenetic Research Workflow

Agilent offers a streamlined, fully customizable solution for detecting copy number changes (CNCs), copy-neutral loss of heterozygosity (LOH), and uniparental disomy (UPD) with our SurePrint G3 CGH+SNP microarrays. Agilent CytoGenomics software is designed to streamline the cytogenetic analysis workflow down to five minutes analysis time for a normal constitutional sample. Agilent CytoGenomics offers a fully automated workflow from sample upload to analysis, an intuitive user interface for manual sample triage, and easily-generated analysis reports. Most importantly, the sample analysis workflow is customizable: users can pre-set parameters with their choice of analysis settings, report settings, aberration classifications, and customized tracks.



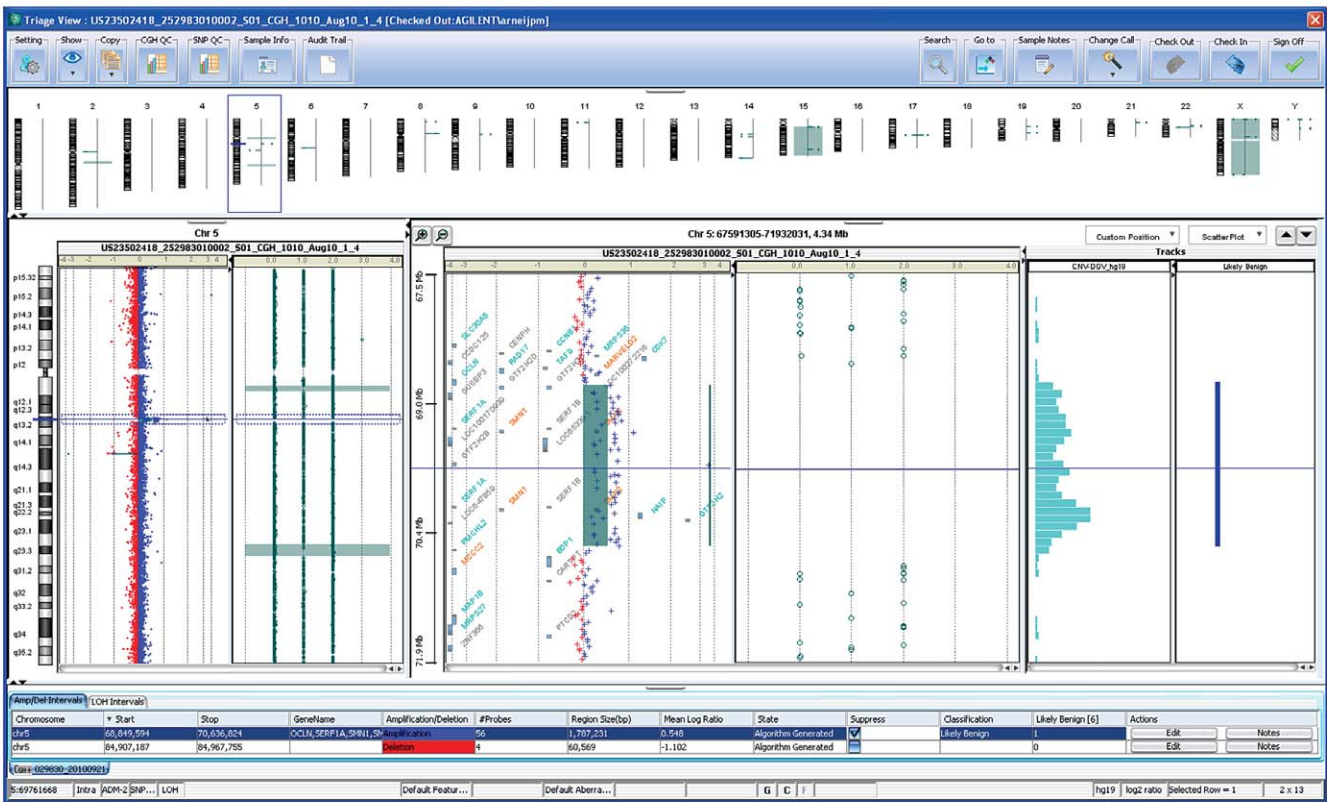
**Agilent Technologies**

## Data Import and Analysis

Data can be imported via TIFF images generated on an Agilent microarray scanner, including the SureScan Microarray Scanner. Agilent CytoGenomics has Feature Extraction (FE) software built in and is able to run FE as an integral part of the analysis workflow. To import the data, the researcher simply selects the proper analysis workflow, points to the file to import, and adds the sample attribute information to map the sample to the array. The sample import process can be done manually or via an automated process called Auto Processing, which is described in more detail below.

## Manual Sample Analysis in the Triage View

Within the Agilent CytoGenomics Triage View, several tools are available to help with manual sample analysis. CNC and LOH/UPD aberration calls can be manually inspected in the Genome, Chromosome, and Gene View (Figure 1). Aberration calls can be classified, suppressed, annotated, or modified based on information available in the Agilent CytoGenomics Database, in which all sample information is stored, or from external databases. Aberration information available in the Agilent CytoGenomics Database can be accessed from dynamic tracks shown in the track view and from within the aberration table. From the Gene View, users can link out to the UCSC Genome Browser and the Database of Genomic Variants (DGV) for further annotation information about genetic areas of interest. Additional information about genes associated with specific aberrations can be obtained by linking out from genes to OMIM, UCSC, DGV, and Entrez.



**Figure 1.** Aberration calls can be manually inspected in the Genome, Chromosome, and Gene View. In this example an ~1.8 Mb amplification (56 probes) on chromosome 5 overlaps with known CNVs present in DGV (Database of Genomic Variants). An overlapping aberration was also observed in a previously analyzed sample stored in the Agilent CytoGenomics Database, and this information is displayed both in the 'Likely Benign' dynamic track and the 'Likely Benign' column in the aberration table. This call can be classified by the researcher as shown in the figure. In addition, the researcher can decide to suppress this call such that it will not be included in the final Cyto Report (suppressed calls are not deleted from the sample, they remain stored in the CytoGenomics Database).

# Cyto Report

Following manual inspection of the sample in the Triage View, the researcher can generate a Cyto Report, which is customizable. The Cyto Report contains information about key aberrations, including the chromosomal location of the aberration, as well as associated genes and classifications. In addition, researchers can include images of the aberration, display notes associated with the aberration or the sample, and include any sample attribute that is available from the database. For example, the Cyto Report in Figure 2 shows a sample with a deletion event on chromosome 17.

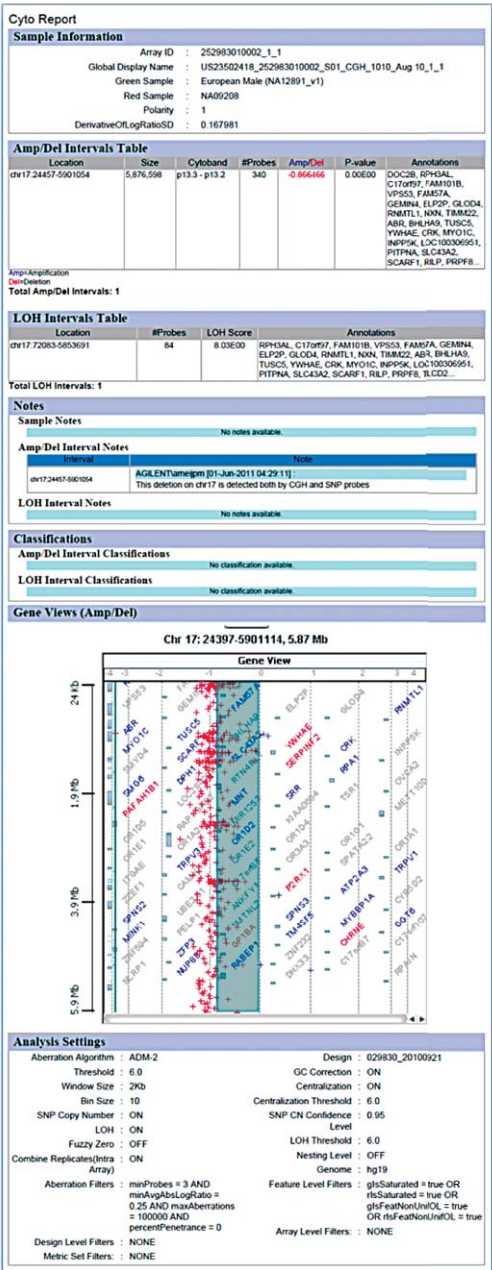


Figure 2. This Cyto Report shows a deletion event on chromosome 17.

# Auto Processing of Data Upload, Feature Extraction, and Data Analysis

Agilent CytoGenomics software supports full automation of data upload, Feature Extraction, and analysis of the FE data by the appropriate CGH and SNP calling algorithms using the Auto Processing feature. When Auto Processing is engaged, the software will automatically import and analyze data from TIFF images that the scanner deposits in a designated folder. This allows the cytogenetic researcher to focus on the manual analysis of the sample within the Triage View. Auto Processing can be easily set up in two steps by i) linking a particular array design with a workflow that can be pre-defined in the software settings and ii) pointing to the location from where to upload both the scanner-generated TIFF image and the text file containing the sample identification and array mapping information. To engage the Auto Processing functionality, simply press the play button in the Auto Processing section in the main UI (Figure 3). The user can now walk away from the system or close the software and it will keep processing data in the background as the TIFF images are being produced by the Agilent scanner. In Auto Processing mode, the user only has to open the software to perform manual triage of the sample and produce an analysis report when analysis is complete.



Figure 3. Agilent CytoGenomics Auto Processing feature.

# Customization of the Sample Analysis Workflow

Agilent CytoGenomics is a workflow-based software. The workflows can be programmed for specific data types and will import the data, run Feature Extraction, run analysis algorithms, and generate results and report files as specified. Default workflows enable the analysis of CGH data, CGH+SNP data, and re-analysis of previously analyzed data from either TIFF images or FE files. In addition, customized workflows—these can include preferred analysis algorithms with custom filters and thresholds—can be generated. Finally, the layout and content of the Cyto Report is also customizable.

## Agilent CytoGenomics Database

The Agilent CytoGenomics Database stores all analyzed sample information. With sample version control, multiple users can access the shared database simultaneously and view the same sample but only one user at a time will be able to manually make changes to a particular sample. To ensure sample version control, the user needs to check out a sample from the database before any manual changes can be made. Other users can view this sample, but they will not be able to make manual changes to it until the first user has checked the sample back into the database. In addition, when investigating an aberration in a sample, the user can query the database to find overlapping aberrations in previously analyzed samples that are stored in the database. Aberration data stored in the Agilent CytoGenomics Database can also be shown in dynamic tracks, along the Gene View within the Triage View. The user can also search the database to retrieve samples with specific sample attribute information, such as age or gender.

## CGH + SNP Data Analysis

Agilent CytoGenomics supports both analysis of CGH and CGH+SNP microarrays. The SNP probes included on the Agilent Sureprint G3 CGH+SNP microarrays can be used to confirm CGH calls and to detect cnLOH or UPD. An example for each application is discussed below.

### Confirming CGH aberration calls with SNP data

Figure 4 illustrates how the Agilent ADM-2 algorithm calls a large deletion on the p arm of chromosome 17 spanning 340 CGH probes using Agilent's SurePrint G3 Human 4x180K CGH+SNP Microarray. This single copy deletion has a mean  $\log_2$  ratio of -0.866. The LOH calling algorithm shows that in the same region there are only single A or B SNP alleles (0 or 1 uncut) instead of the combination of AA, AB, and BB SNP alleles (0, 1, and 2 uncut alleles) present in a normal diploid genome (Figure 4). For more information about the SNP and LOH calling algorithms see the application note, *Simultaneous Detection of Copy Number and Copy-Neutral LOH Using a Single Microarray* (P/N 5990-6274EN).

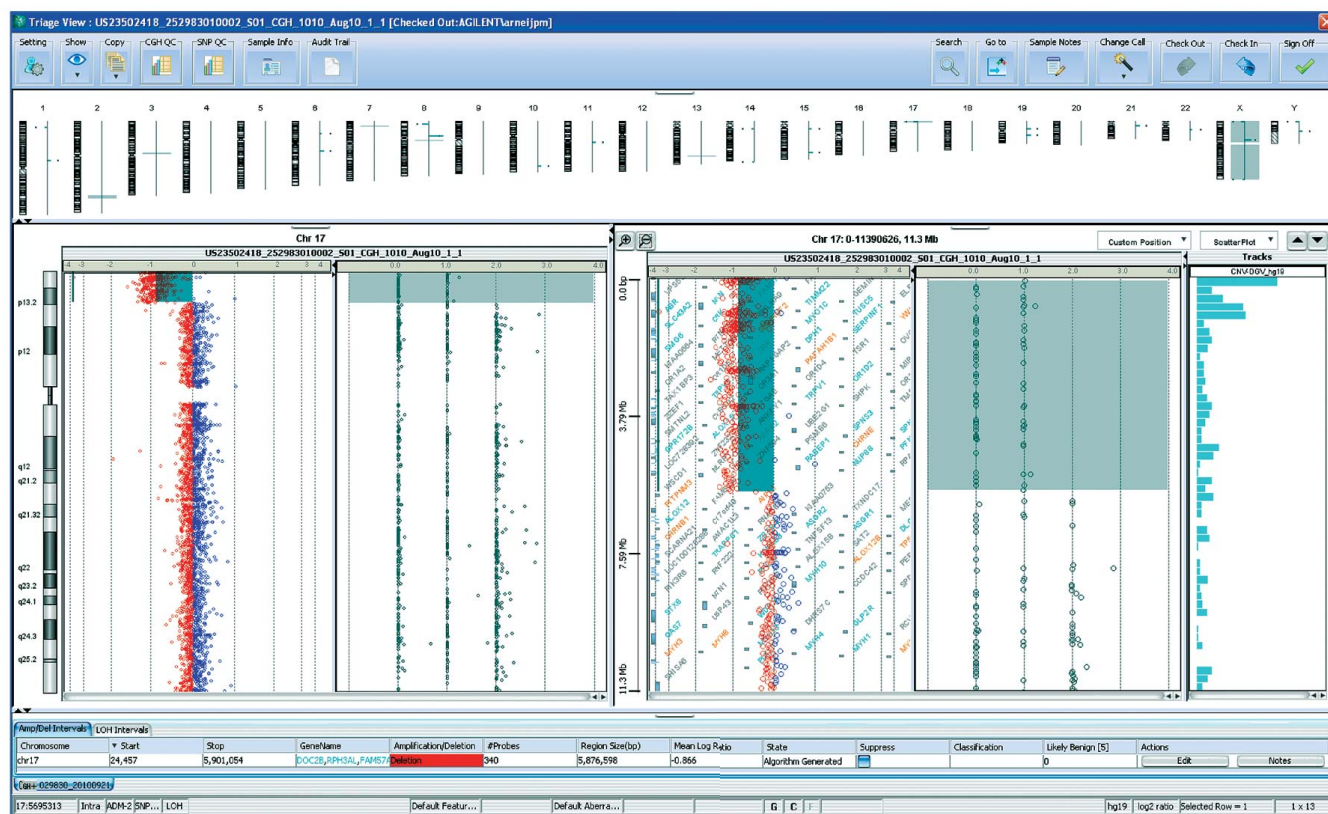


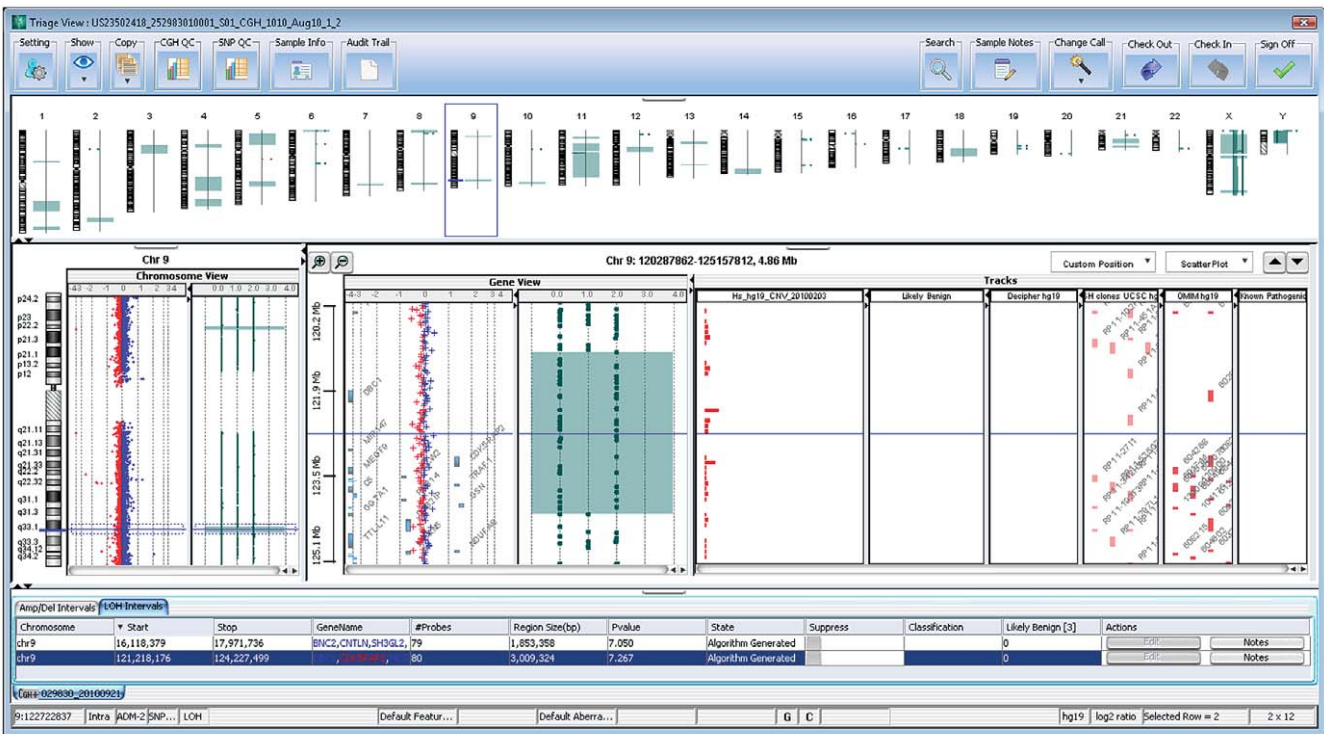
Figure 4. Calling a deletion. This figure shows the ability of Agilent's CGH+SNP microarray to call copy number aberrations using both CGH and SNP probes. In this sample, a deletion is detected on chromosome 17.



LOH calls in a consanguineous sample

A consanguineous sample has related parents. As a result the genome can have long stretches of copy-neutral (cn) LOH. This sample illustrates the ability of the Agilent CytoGenomics software to call LOH events as low as 5–10 Mb across the genome. An example of this low-level LOH detection is shown on chromosome 9, where a 3 Mb LOH event with a significant confidence level of 7.26 (which is higher than Agilent’s suggested cut-off value of 6) is called (Figure 5).

This cnLOH event is detected by the SNP probes as a loss of the single uncut alleles, corresponding to a loss of the heterozygous SNP calls. The CGH probe plot, however, looks completely normal for this interval, making the additional SNP probes on the array an invaluable addition for detecting copy-neutral LOH events.



**Figure 5.** Consanguineous sample. The ability of Agilent CGH+SNP microarrays to detect low-level LOH is shown in this sample. On chromosome 9, a 3 Mb LOH event with a significant confidence level of 7.26 is called.

## System Setup

Agilent CytoGenomics is a client/server system. Data analysis is processed on the client while the primary function of the server is to host the database and its content. Agilent CytoGenomics client and server can be installed on one machine. They are supported on both 32- and 64-bit systems

and compatible with Windows XP and Windows 7 for PC and Mac OS 10.5 and 10.6. Alternately, the system can be set up with one central database server that multiple clients can connect to simultaneously. For this configuration, the client can be either a PC or Mac and be either 32- or 64-bit, but the server has to be installed on a PC that is either 32-bit or 64-bit (Figure 6).

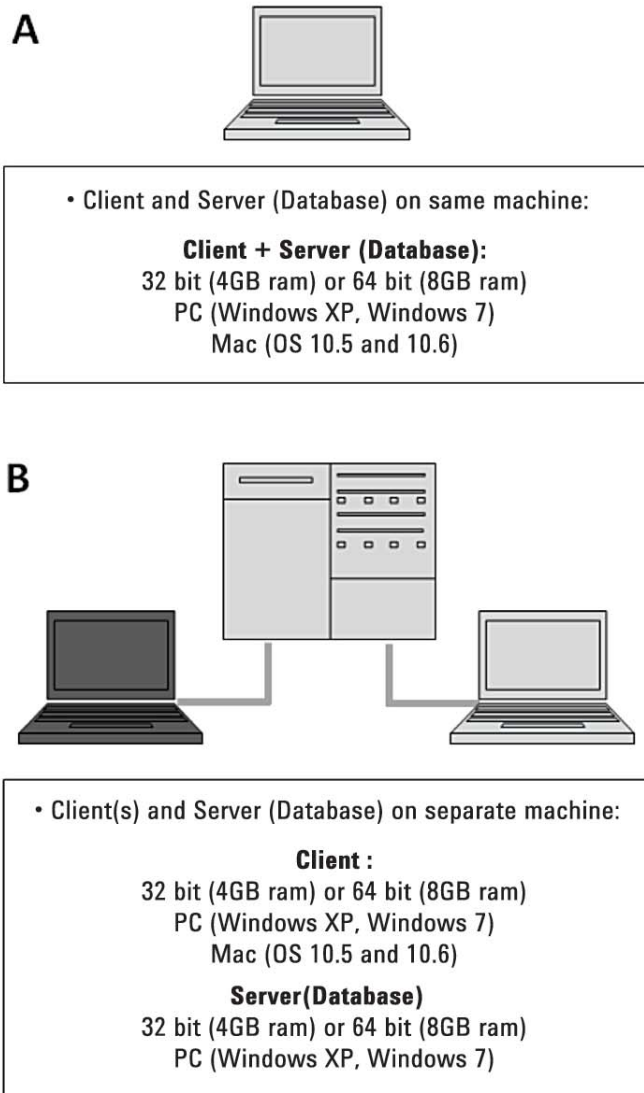


Figure 6. System setup on 32- and 64-bit systems with client and server installed on one machine or on one server with multiple clients.

## **Conclusion**

Agilent CytoGenomics software, designed for the detection and analysis of copy number and copy-neutral chromosomal aberrations, is a powerful addition to Agilent's leading Sureprint G3 CGH+SNP and CGH microarray platforms. Integrating manual sample analysis with auto processing and customized workflow design, Agilent CytoGenomics completes Agilent's cytogenetic research solution. With Agilent CytoGenomics, calling copy number and LOH aberrations is simpler, more efficient, and more accurate.

## Agilent CytoGenomics Products

Description	Part Number
Agilent CytoGenomics Annual license for 1 client/ server installation	G1662AA

[www.agilent.com/genomics/cyto\\_software](http://www.agilent.com/genomics/cyto_software)

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Agilent Technologies, Inc. 2011  
Published in USA, September 2, 2011  
Publication Number 5990-8533EN



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