

Automating the CGH/CNV Workflow with the Bravo Automated Liquid Handling Platform

Application Note

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Abstract

The Agilent Comparative Genomic Hybridization (CGH) and Copy Number Variation (CNV) portfolio combines flexibility and sensitivity by providing catalog and custom microarrays designed specifically for researchers studying aberrations and copy number variations in numerous organisms. To take advantage of the true power of the Agilent CGH/CNV system, researchers need a platform that provides high throughput with minimal operator intervention, without sacrificing accuracy or sensitivity. A new method was developed to automate CGH/CNV sample fragmentation, labeling, purification, and pre-hybridization setup in a 96-well format by using the Agilent Bravo Automated Liquid Handling Platform. Protocols for both the standard enzymatic (Genomic DNA Enzymatic Labeling Kit) and ULS (Genomic DNA High-Throughput ULS Labeling Kit) methodologies have been developed and validated with VWorks Automation Control software. The new workflow, compatible with both SurePrint HD and G3 microarrays, enables superior performance across the entire Agilent CGH/CNV portfolio. Throughput is increased and hands-on time is decreased, with no compromise in data quality.



Introduction

As CGH/CNV microarrays are becoming a more widespread tool used in various research applications, there is a need for a system that is automated and easier to use. The Bravo Automated Liquid Handling Platform (**Figure 1**) provides unparalleled speed, versatility, and precision in a compact footprint. The Bravo Platform uses proven high-accuracy pipette heads and its standard 96- and 384-channel heads can access a single column, row, or well of a microtiter plate. The Bravo Platform is powered by VWorks Automation Control software. This industry-leading software features outstanding dynamic scheduling, easy-to-use interface, and innovative error-recovery technology, providing complete control to operators of all levels. More information on the Bravo Automated Liquid Handling Platform is available at www.agilent.com/lifesciences/automation and in the Data Sheet (5990-3480EN). In this Application Note, we will demonstrate the performance of the Bravo Automated Liquid Handling Platform for the Agilent CGH/CNV workflow using two different labeling methodologies: enzymatic and ULS. The enzymatic method uses exo-Klenow to extend from priming sites and incorporate labeled dUTPs while the ULS method is a platinum-based labeling technology that allows for the direct coupling of Cy3 or Cy5 to DNA. The two Agilent reagent kits available for use with the Bravo Platform and CGH/CNV microarrays are the Genomic DNA Enzymatic Labeling Kit (5190-0449) and the Genomic DNA High-Throughput ULS Labeling Kit (5190-0450). The Agilent CGH/CNV Workstations come preconfigured from the factory with the proper deck setup and protocols.

- Pre-configured Bravo; Enzymatic G5409A Option 002
- Pre-configured Bravo; ULS G5409A Option 003

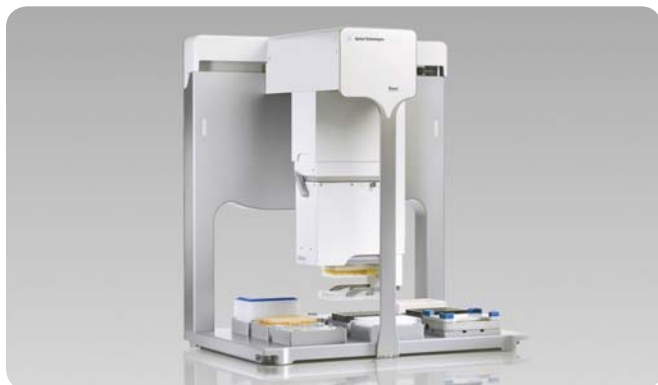


Figure 1. Agilent Bravo Automated Liquid Handling Platform.

Overview of Protocol

Figure 2 illustrates the complete CGH workflow for both the enzymatic and ULS methods for sample preparation and microarray processing. Steps that are in blue use the Bravo Automated Liquid Handling Platform. For a more detailed look at the workflow, see the Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis Bravo Automated Liquid Handling Platform with Enzymatic and ULS Labeling version 1 User Manual available at <http://www.agilent.com/chem>. VWorks Automation Control software provides three protocols each for ULS processing and enzymatic processing: Fragmentation Labeling Protocol, Purification Protocol, and Hybridization Prep Protocol. The user can input the desired number of samples (from 1 to 48) and array format (1x, 2x, 4x, or 8x). The Agilent Oligo aCGH Bravo Platform protocols are available on the CD that comes with the Bravo platform or may be obtained by contacting your local Agilent Product Specialist. These protocols require only 60 minutes of hands-on time to process 48 samples on the 4x180K microarray (**Table 1**).

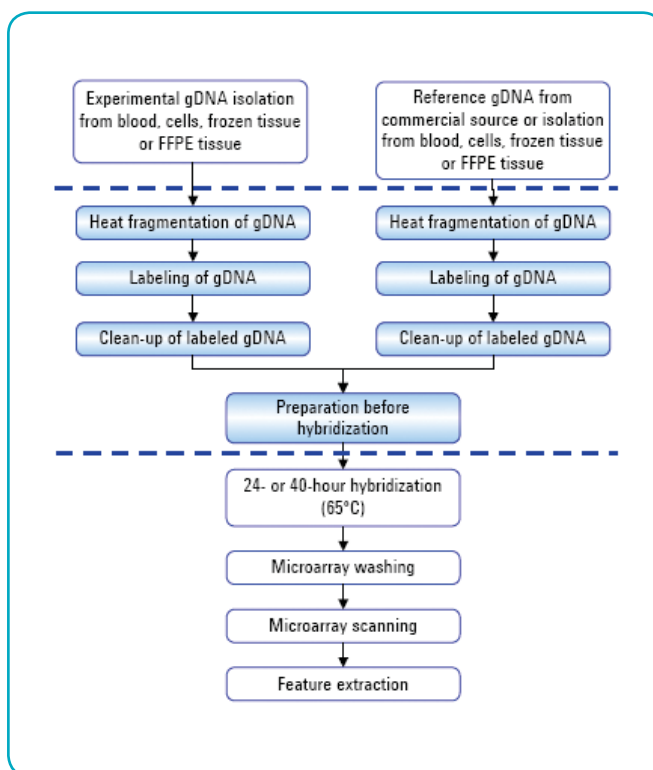


Figure 2. Workflow diagram for both the enzymatic and ULS methods for sample preparation and microarray processing. Steps in blue use the Bravo Automated Liquid Handling Platform.

Biological Results

Reproducibility

A key requirement for an automated system is reproducibility. To test the reproducibility of this system, an entire 96-well plate containing 48 replicates of the same sample (500ng Promega Male) and the same reference (500ng Promega Female) was processed on the Bravo Automated Liquid Handling Platform using

the enzymatic labeling option. Six Male/Female sample pairs were randomly selected and hybridized on the Agilent SurePrint G3 Human Catalog CGH 4x180K Microarrays (G4449A). The yield and specific activities (pmol dye per µg DNA) were measured on an 8-channel NanoDrop after labeling and purification, and microarray QC metrics were obtained in Genomic Workbench 5.0 (**Table 2**). The standard deviations of the yields, the specific activity and the microarray QC metrics were very

Time (48 samples)	Fragmentation & Labeling	Purification	Hybridization	Wash & Scan
Hands-on	15 minutes	5 minutes	10 minutes	30 minutes
Bravo Platform/Scanner	25 minutes	5 minutes	25 minutes	2 hours 25 minutes
Centrifugation	4x30 seconds	3x5 minutes	30 seconds	
Incubation *enzymatic labeling **ULS labeling	2 hours 20 minutes* or 40 minutes**	NA	24 hours 35 minutes	3x6 minutes

Table 1. Processing of 48 samples on 4x180K microarrays requires only 60 minutes of hands-on time.

Metric	Average +/- Std Deviation
Yield (µg)†	5.4±0.2
SpecificActivityGreen (pmol/µg) †	17.4±0.7
SpecificActivityRed (pmol/µg) †	16.8±0.6
DLRSpread*	0.13±0.003
Signal-to-Noise Green*	87.8±3.8
Signal-to-Noise Red*	125.8±8.8
Reproducibility Green*	6.7±1.2
Reproducibility Red*	6.4±1.1
AreaUnderROC*	0.97±0.002
MedianDiff*	-0.96±0.01

Table 2. The table shows the yield and specific activity results for 48 normal female and normal male samples. In addition, the microarray QC metrics for 6 normal female and male samples processed on the Bravo Automated Liquid Handling Platform (500ng input, enzymatic labeling option) and hybridized on Agilent SurePrint G3 Catalog CGH 4x180K Microarrays (G4449A) are shown.

† n=48; *n=6

small, indicating the superior reproducibility of this system. In the Male/Female (XY/XX) model system, X-chromosome probes have a theoretical \log_2 ratio of -1 (MedianDiff in Table 2). We show that the \log_2 ratios obtained were close to the expected values (-0.96). Further, there is an excellent separation between autosomal probes and X-chromosome probes indicated by an Area under ROC curve of 97% and a small standard deviation across the samples.

Comparison with Manual Processing

Enzymatic labeling

In configuring the enzymatic protocols for automation, several steps were optimized. The restriction digestion was replaced by a longer denaturation/fragmentation step in the presence of the random primers, and the labeling reaction volume was decreased. To ensure that these modifications had minimal

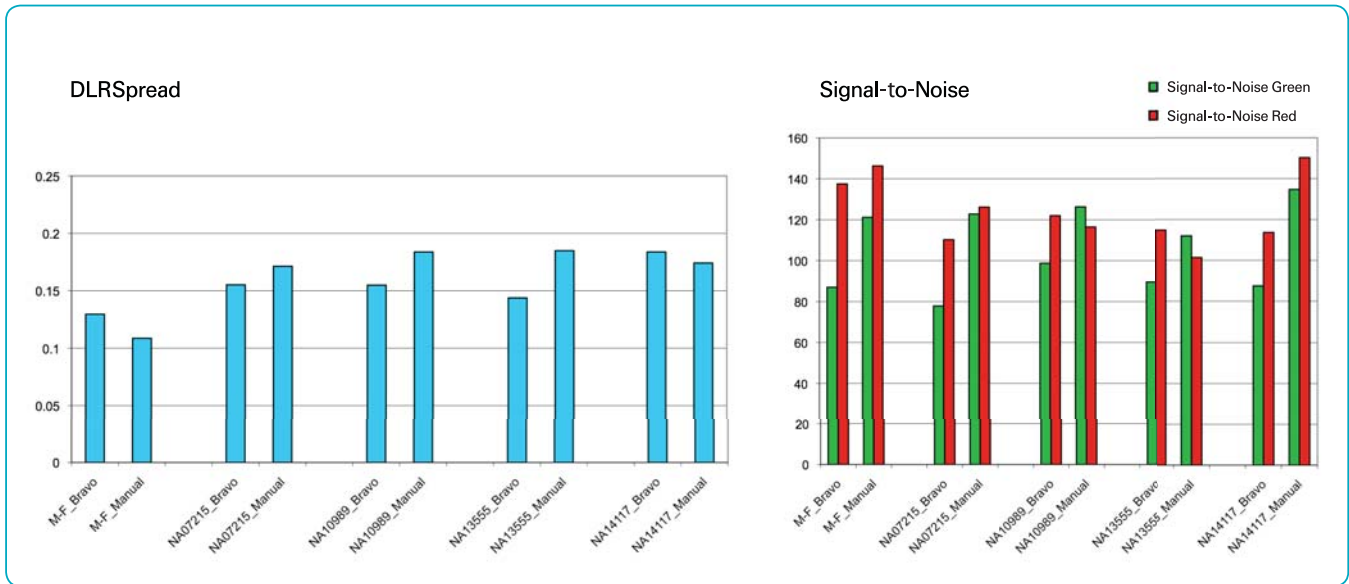


Figure 3. DLRSread and Signal-to-Noise metrics for five different samples. Promega Male, NA07215, NA10989, NA13555, and NA14117 (reference Promega Female), processed in either manual or Bravo platform automated mode (500ng input, enzymatic labeling option) and hybridized on Agilent SurePrint G3 Catalog CGH 4x180K Microarrays (G4449A).

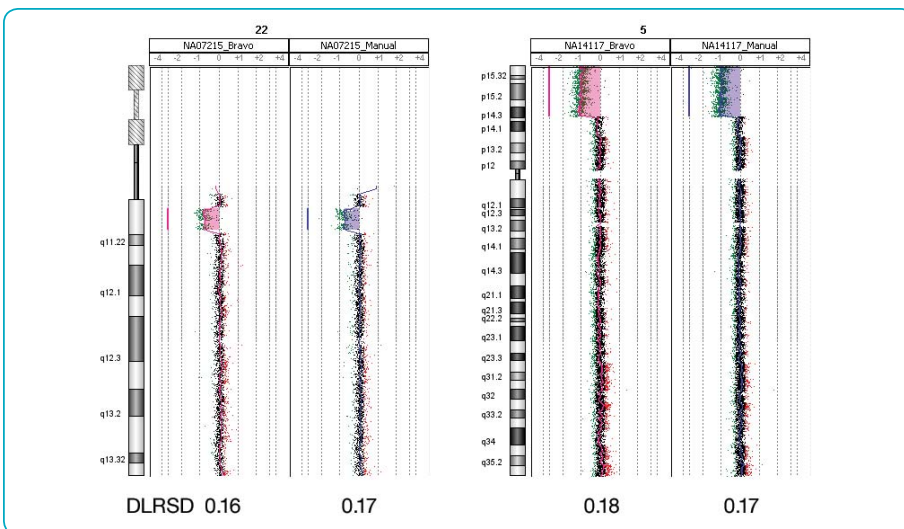


Figure 4. Comparison of Copy Number Profiles. Using DNA processed (500ng input, enzymatic labeling option) on the Bravo Automated Liquid Handling Platform (pink) or manually (blue) and hybridized on Agilent SurePrint G3 Catalog CGH 4x180K Microarrays (G4449A). Genomic Workbench 5.0 settings: 1 Mb moving average, ADM-2, threshold 5, filter: 2 probes, 0.25 \log_2 ratio.

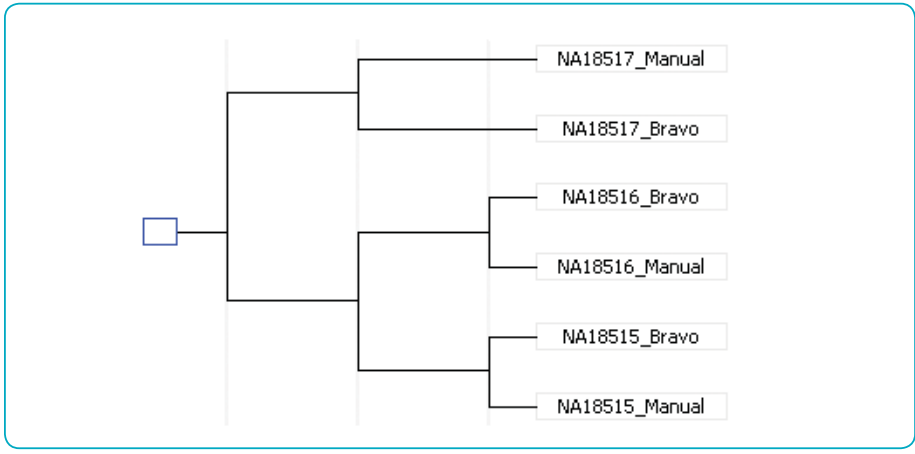


Figure 5. Clustering of a trio of HapMap samples: Yoruban mother (NA18517), father (NA18516), and child (NA18515)(reference NA15510). Processing was performed on the Bravo Automated Liquid Handling Platform or manually (1000ng, ULS labeling option) and hybridized on CNV Association 2x105K Microarrays (G4417A). Genomic Workbench 5.0 hierarchical tree clustering algorithm settings used: Distance Manhattan, Linkage Single, Use Weighted Distance, Tree Level 5, Use Aberration Calls.

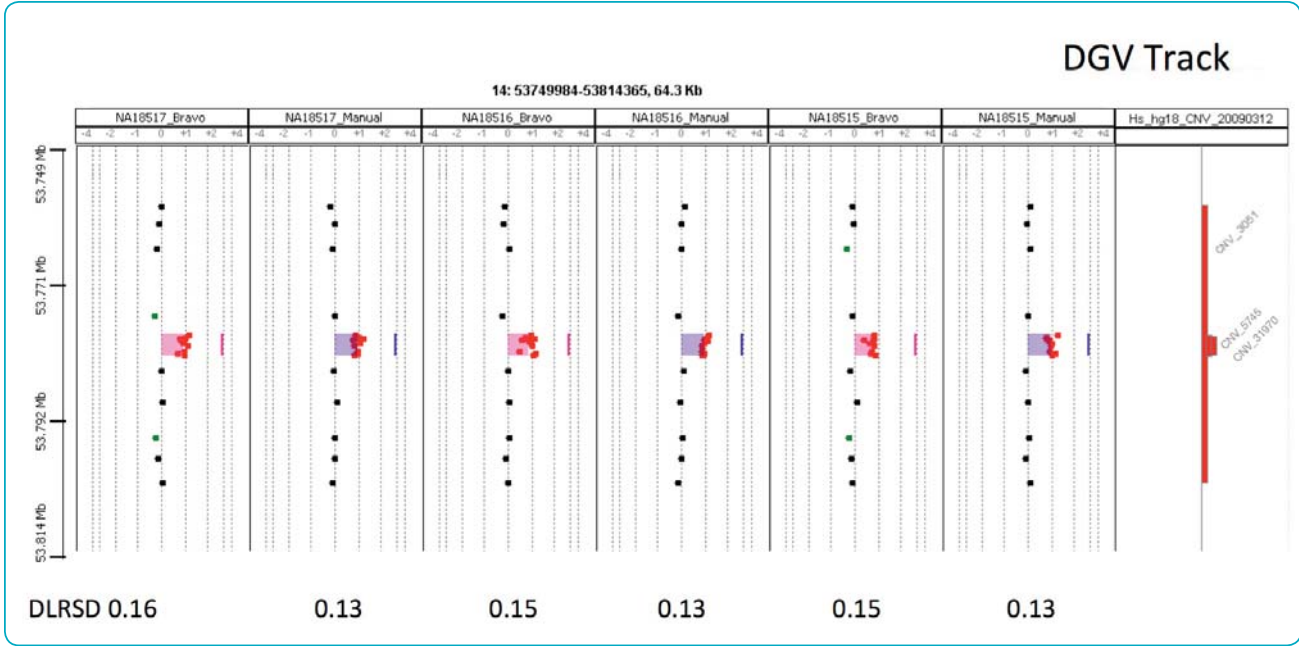


Figure 6. Confirmation of a known CNV (3.5 Kb) on Chromosome 14 in a trio of HapMap samples: a Yoruban mother (NA18517), father (NA18516), and child (NA18515). Processing was performed on the Bravo Automated Liquid Handling Platform (pink) or manually (blue) (1000ng, ULS labeling option) and hybridized on CNV Association 2x105K Microarrays (G4417A). Genomic Workbench 5.0 settings: 1 Mb moving average, ADM-2, threshold 5, filter: 2 probes, 0.25 log₂ ratio, track Agilent_022837_Regions used as genomic boundaries.

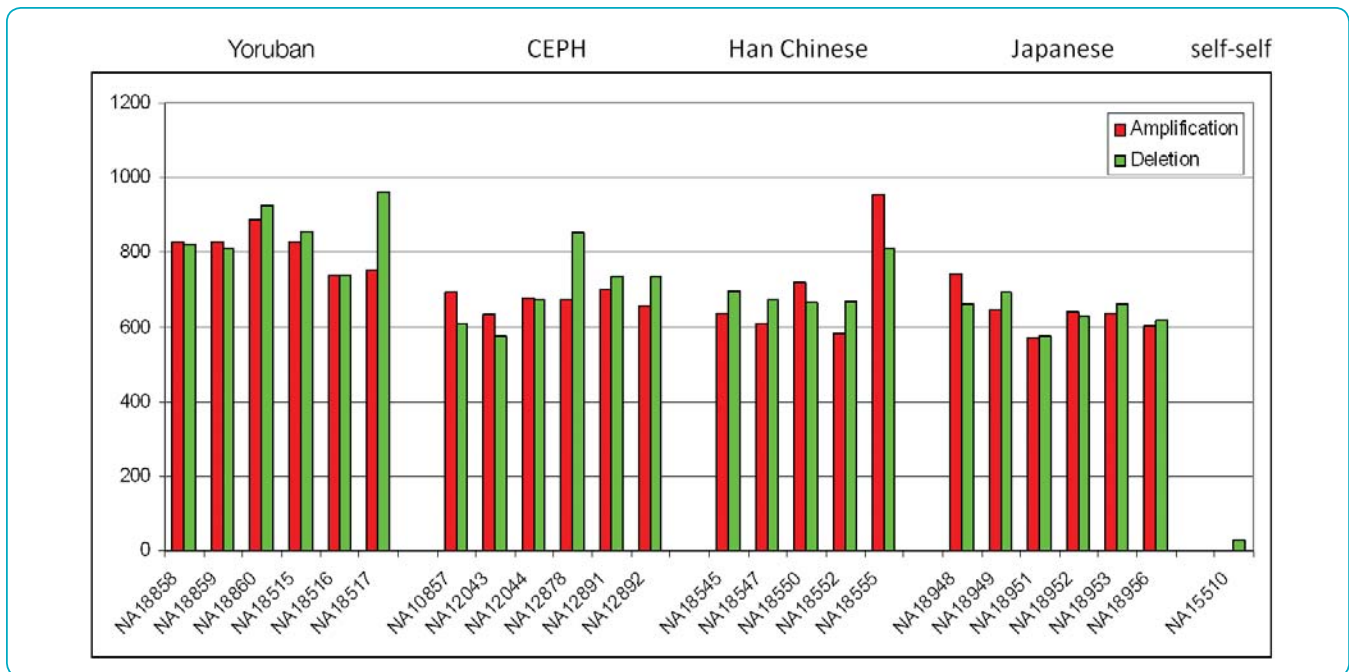


Figure 7. Number of CNVs in 24 different HapMap samples (NA15510 as reference). Samples were processed on the Bravo Automated Liquid Handling Platform (1000ng, ULS labeling option) and hybridized on CNV Association 2x105K Microarrays (G4417A). Genomic Workbench 5.0 settings: 1 Mb moving average, ADM-2, threshold 5, filter: 2 probes, 0.25 log₂ ratio, track Agilent_022837_Regions used as genomic boundaries.

impact on CGH data, an experiment was performed to compare manual processing without protocol adjustments with the automated mode including new optimized steps. In this experiment, several different samples were run and the QC performance metrics and array results were compared. **Figure 3** shows that the DLRSread (probe-to-probe log ratio noise) and the Signal-to-Noise values across five different sample sets are equivalent for manual and automated processing.

Most importantly, the array results from the Bravo Platform are identical to the manually processed samples (**Figure 4**). The aberrations associated with DiGeorge Syndrome and Cri-du-Chat on chromosome 22 of sample NA07215 and chromosome 5 of sample NA14117, respectively, were detected with both methods.

ULS Labeling

Only very minor changes were made to the ULS manual labeling protocol to make it more amenable for automation. Indeed, when comparing a trio of HapMap samples processed manually or on the Bravo Automated Liquid Handling Platform and hybridized to the CNV Association 2x105K Microarrays (G4417A) the data clustered together by sample and not by processing method (**Figure 5**). An example of a known CNV on

chromosome 14 (reported in the Database of Genomic Variants <http://projects.tcag.ca/variation>) is shown in **Figure 6**.

CNV Pilot Study

A large sample set was run on the Bravo Automated Liquid Handling Platform. The experiment used the ULS labeling method, and a set of 24 different HapMap samples obtained from Coriell (<http://ccr.coriell.org/Sections/Collections/NIGMS/?Sslid=8>) was hybridized against NA15510 on the CNV Association 2x105K Microarrays (G4417A). The average DLRS D was 0.15 with a standard deviation of 0.01 and the average Green Signal to Noise (from the same reference sample) was 68.14 with a standard deviation of 8.95, indicating excellent, consistent results. **Figure 7** shows the number of aberrations obtained for all samples. The number of aberrations in the self-self was very low, indicating a low rate of false positives. As expected, because the reference sample NA15510 appears to be of European descent (Korbel et al. Science 2007 Oct 19;318(5849):420-6), the number of aberrations for the CEPH (Centre d'Etude du Polymorphisme Humain) samples NA10857 through NA12892 is lower as compared to the Yoruban samples NA18858 through NA18517, when hybridized against NA15510.

Conclusions

We have demonstrated an effective new method for automating CGH/CNV sample fragmentation, labeling, purification, and pre-hybridization setup in a 96-well format with the Bravo Automated Liquid Handling Platform. The automated workflow showed excellent reproducibility and yielded results that were consistent with manual processing. Hands-on time was reduced and throughput was increased for both the standard enzymatic and ULS protocols without compromising data quality. These robust, ready-to-go, validated protocols allow you to start automating your CGH workflow immediately, with no setup or programming required. Automating Agilent's CGH/CNV workflow produces the throughput necessary for researchers to process samples accurately—faster and with minimal operator intervention.

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