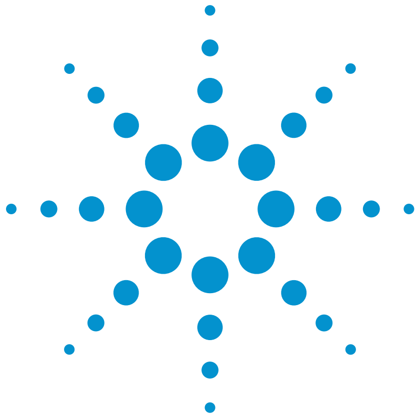

Agilent CGH+SNP Microarrays Demonstrate Superior Copy Number Calling for Cytogenetic Profiling of Hematological Cancer Samples

Application Note

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Introduction

Recent comparative studies of high-resolution copy number platforms show that platforms with long oligonucleotides consistently outperform the SNP platforms with regard to copy number analysis.[1, 2, 3, 4] SNP microarrays were developed for linkage analysis for the identification of single nucleotide polymorphisms (SNPs) and allow for the simultaneous detection of DNA copy-neutral loss of heterozygosity (LOH). Agilent has added specific SNP content to the Agilent CGH+SNP microarrays to offer the advantage of simultaneous determination of high resolution DNA copy number changes and LOH. In this application note, we compare the quality of copy number and LOH data obtained with Agilent CGH+SNP microarrays versus the quality obtained with Illumina SNP microarrays using DNA isolated from chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) samples.

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Experimental

Two commercially-available, catalog, high-resolution oligonucleotide microarray platforms, the Agilent SurePrint G3 CGH+SNP 2×400K microarray platform and the Illumina HumanCytoSNP-12v1 were compared. Information and characteristics for each platform are summarized in Table 1.

Six samples were selected for a comparison between the two platforms. DNA was labeled and hybridized according to the manufacturers' instructions. The same DNA preparations were used for analysis on both platforms to avoid the effects of DNA quality or sample variability on the results. Data processing and analysis were performed using the corresponding software for each of the platforms, Agilent CytoGenomics and Illumina KaryoStudio, using the default settings. Platform-independent copy number analysis was also performed in Nexus (BioDiscovery) software. The algorithms in the Nexus software are not optimized to analyze Agilent SNP data from cancer samples.

Table 1. Microarray platform characteristics.

Platform	Oligonucleotide length	Sample labeling	Sample requirement	Number of CGH features	Number of SNP features
Agilent SurePrint G3 CGH+SNP 2×400K microarray platform	60 nt	Direct labeling	500 ng	292,097	118,955*
Illumina HumanCytoSNP-12v1	25 nt	WGA amplification	200 ng	0	299,671

*92 % of SNPs use two SNP probes per SNP

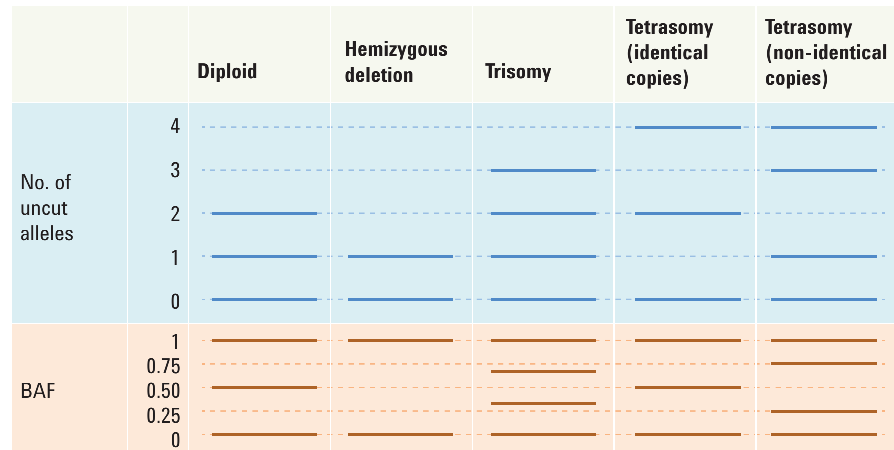

Figure 1. Comparison of number of uncut alleles and B-allele frequency (BAF).

Results and Discussion

B-allele frequency (SNP array) versus number of uncut alleles (CGH+SNP array)

Illumina SNP data is typically visualized using B-allele frequency (BAF) plots. However, with the Agilent CGH+SNP microarrays, the number of uncut alleles and total copy number are measured instead of the A and B allele frequency.[5, 6] Accordingly, the Agilent SNP data is visualized as the number of uncut alleles (Figure 1). In diploid regions of the genome, the BAF and number of uncut allele plots look very similar. In amplified or deleted regions, the plots look different. This is because the scale for displaying the homozygous (AA, BB) and heterozygous alleles (AB) is always from 0 to 1 in Illumina BAF plots versus from 0 to 1, 2, 3,... in Agilent SNP copy number plots. As a result, it is not possible to distinguish a tetrasomy where both the maternal and paternal chromosomes were amplified from a diploid region of the genome in BAF plots. Moreover, when looking at BAF, especially in mosaic samples (aberrant sample diluted with normal), it is more difficult to distinguish a trisomy (BAF = 0 %, 33 %, 66 %, 100 %) from a tetrasomy where only one parental chromosome was amplified (BAF = 0 %, 25 %, 75 %, 100 %).

Detection of chromosomal aberrations in mosaic samples

Cancer samples typically consist of a cell pool containing cells with normal karyotype and cells with chromosomal aberrations. Valli *et al.* have reported the ability to detect levels of abnormal cells as low as 8 % in constitutional and acquired mosaicism on Agilent CGH microarrays.[7] Agilent CytoGenomics software was used to determine the clonal fraction of the six samples used in this study. Clonal fractions varied from <10 % to 87.5 % (Table 2). A subclone (28.3 % of the cells) was found in CLL sample 2. Clonal fractions are not reported in Illumina KaryoStudio or Nexus software.

The most significant genomic aberrations found in the six samples are summarized in Table 2. Consistent with the literature, a deletion in the long arm of chromosome 13 was the most common abnormality. Figure 2 compares the ability of the Agilent CGH+SNP and the Illumina SNP platform to find a deletion on chromosome 13 in mosaic MM sample 6. The deletion was detected in the Agilent copy number (CN) data with both Agilent CytoGenomics and Nexus software. The SNP plot for this sample in the Agilent CytoGenomics software confirmed the hemizygous deletion in this same region. The Agilent CytoGenomics software reported the clonal fraction in this sample as approximately 24.5 %. This was confirmed by FISH where the deletion was found in 69 out of 222 (31 %) cells.

Table 2. Karyotype and/or FISH findings, clonal fraction (calculated in CytoGenomics software), and most significant genomic aberrations.

Sample number	Sample type	Karyotype and/or FISH findings	Clonal fraction	Most significant genomic aberrations
1	CLL	nuc ish (CEP12x2,D13S319x1,13q34x2)[260/413] nuc ish (ATMx1,p53x2)[248/358] nuc ish (IGHx2)[400]	62.3 %	Hemizygous deletions on chr. 11 and 13.
2	CLL	nuc ish (CEP12x2,D13S319x1,13q34x2)[89/200] nuc ish (IGHx2)[200] nuc ish (ATMx2,p53x1)[93/200]	48.5 % Subclone 28.3 %	Heterozygous deletions of variable size on chr. 4, 5, 6, 13, 17, and 18. Subclone: additional terminal deletion on chr. 18.
3	MM	42,X,-Y,add(1)(p13),add(6)(q2?2),add(11)(q13),-13,-14,add(14)(q32),-22[3]/46,XY[12]	13.3 %	Copy number loss of chr. 13, 14, 22, and Y. Extra copy of the distal part of chr 1 and X.
4	CLL	nuc ish (CEP12x2,D13S319x1,13q34x2)[124/200] nuc ish (ATM, p53)x2[200] nuc ish (IGHx2)[200]	87.5 %	Small deletion on chr. 13.
5	MM	46,XX[19] 1 metaphase with 53 chromosomes, 1p+,+3,+4,+5,+9,+11,+15,-16,-18,+19,+19,+19,-20,-21,-22,+3mar nuc ish (D11Z1x3, D13S319x2)[26/200] nuc ish (IGHx2)[200]	<10 %	Copy number gain on chr. 3, 4, 5, 9, 11, 15, and 19.
6	MM	46,XX[20] nuc ish (D11Z1x2,D13S319x1)[69/222](31%) nuc ish (3′IgHx2,5′IgHx1)(3′IgH sep 5′IgH)x1 (22%)	24.5 %	Deletion on chr. 13.

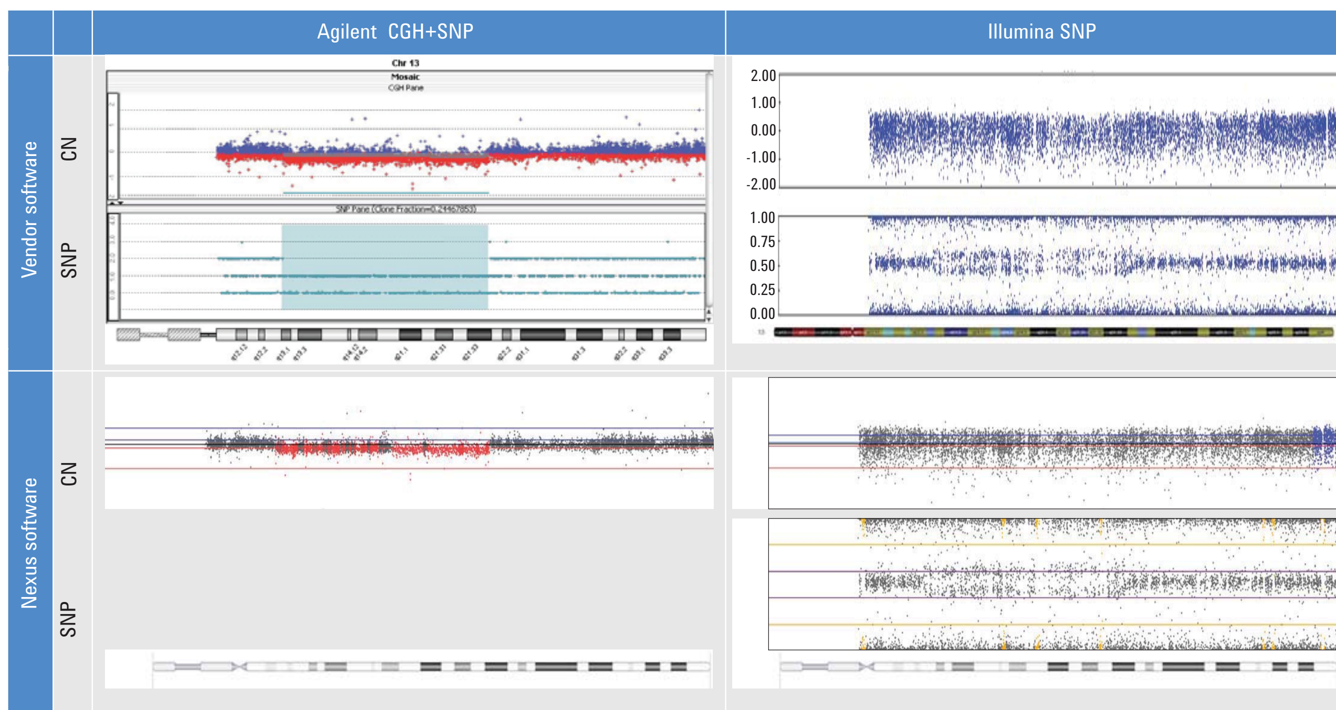

Figure 2. DNA CN and SNP profile of chromosome 13 of MM sample 6 hybridized on Agilent SurePrint G3 CGH+SNP 2×400K microarrays and Illumina SNP microarrays analyzed with the respective vendor or Nexus software. Results indicated that the deletion present in approximately 25 % of the cells could be detected on Agilent's platform in both the CN and SNP data, but was only detectable as an aberration in the Illumina SNP data.

The deletion could not be detected in the Illumina copy number data, either with Illumina KaryoStudio or Nexus software. However, the Illumina SNP data confirmed that an aberration was present in the sample. In addition, the Nexus software reported an amplification on the q arm of chromosome 13 for which there is no evidence in the Agilent CGH or SNP data. This may be a false positive amplification call.

Conclusion

We have shown that the addition of SNP probes to the Agilent SurePrint G3 CGH microarrays and the enhanced analysis algorithms in CytoGenomics software enable the sensitive detection of copy number aberrations and blocks of copy neutral LOH in hematological cancer samples. Visualizing the SNP data as number of uncut allele plots, as compared to B-allele frequency plots, allows for an easier determination of parental origin of amplified or deleted regions. Consistent with previous reports in the literature, the most common aberration found in this set of samples was a deletion on chromosome 13. In a sample contaminated with an approximately 75 % normal cells, the Agilent copy number calls matched the SNP calls in a hemizygously deleted region while the Illumina copy number calls missed this aberration.

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