# Genomic Profiling of LOH and DNA Copy Number with Infinium® Whole-Genome Genotyping

Illumina's Infinium Assays enable the investigation of chromosomal aberrations in both cancer and congenital samples.

## Introduction

Traditionally, array-CGH has been utilized to study genomic alterations underlying cancer initiation and progression. This classical technique involves hybridization of genomic fragments to microarrays from reference and subject samples and analysis of the normalized intensities between these samples. In combination with Illumina's Infinium Whole-Genome Genotyping Assay1 and Sentrix® Whole-Genome Genotyping BeadChips[2], SNP array-CGH supports genomic profiling at unparalleled resolution. Using this approach, the genome can be scanned for DNA copy number changes, loss of heterozygosity (LOH), copy-neutral LOH events, and other chromosomal aberrations characteristically found in cancers and congenital disorders. The combined use of normalized intensity measurements and allelic ratios delivers a more precise profile of chromosomal aberrations across the genome.

## The Importance of Studying DNA Copy Number

Changes in DNA copy number can activate oncogenes or inactivate tumor suppressor genes (TSGs), the root cause of many human cancers. LOH analyses have helped identify many TSGs including RB1, WT1, and TP53, which are involved in the development of retinoblastoma, Wilm's tumor, and Li-Fraumeni syndrome, respectively3. Recently, LaFramboise and colleagues found that most amplifications observed in lung cancer occur as a result of monoallelic amplification4. This discovery has direct consequences for predicting cancer therapeutic efficacy since certain amplifications have been documented to have the best correlation with clinical outcome[5]. In addition, many congenital disorders have been attributed to variations in gene dosage, such as Parkinson's disease, whereby a triplication of the alpha-synuclein locus contributes to the risk of developing the disease[6].

## Illumina's LOH and Copy Number Analysis

With Infinium Whole-Genome Genotyping, chromosomal aberrations are detected by comparing the normalized intensity of a subject sample and a reference sample using two modes of analysis. The first is a single-sample mode in which reference values are derived from canonical genotyping clusters (at 0, 0.5, and 1.0) created from clustering on ~120 normal reference samples. The second is a paired-sample mode in which direct intensity (R) comparisons between a subject sample and its paired reference sample are performed. The basis for detecting chromosomal aberrations are genomic plots of the log2 (Rsubject/Rreference; log R ratio) and the allele frequency (AF) parameters, which are the known B allele frequencies (or fractions) of the three canonical clusters.

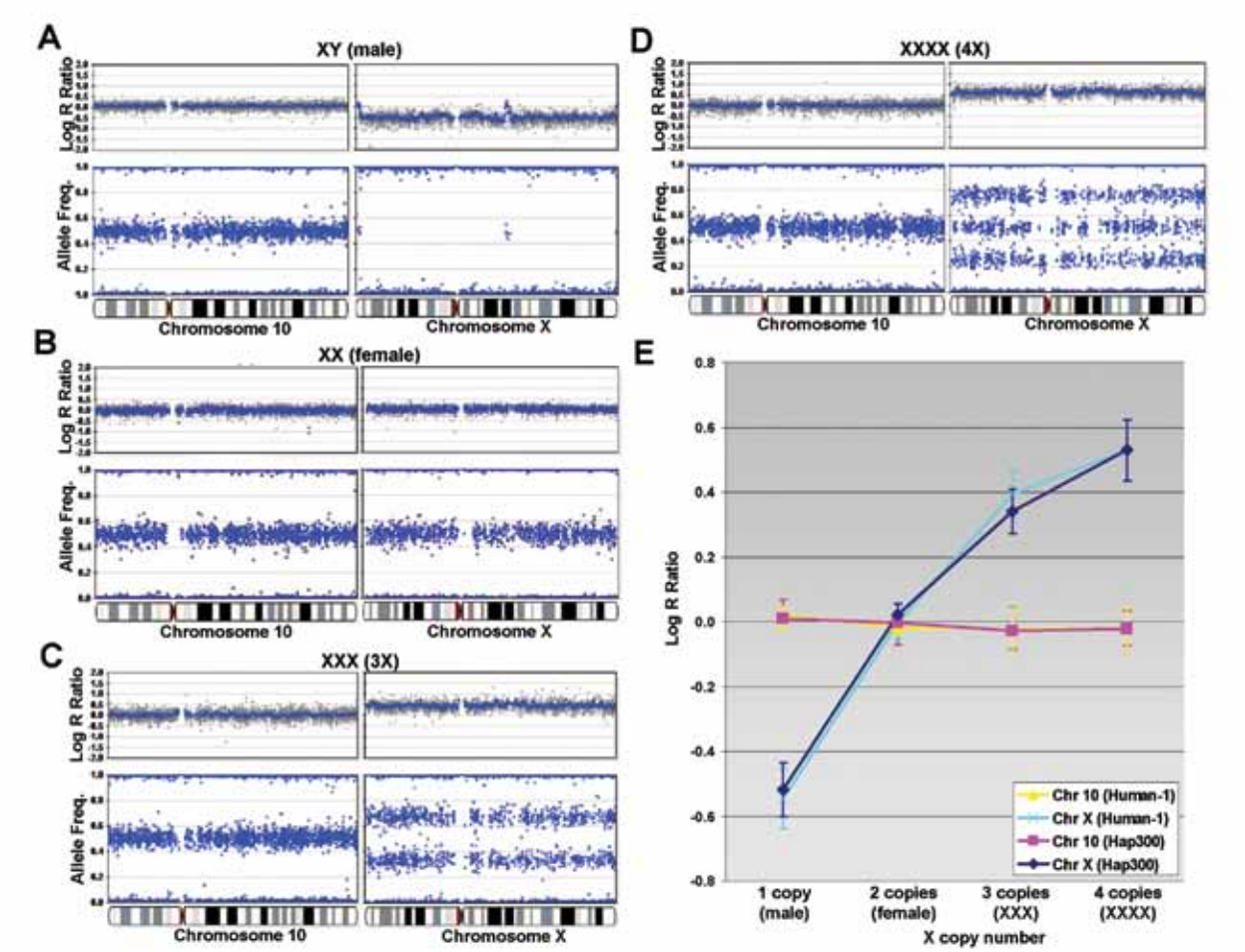
## Ability to Detect Changes in DNA Copy Number

The ability to detect single changes in DNA copy number with low variability levels is of paramount importance in cancer biology. A model system consisting of cell lines containing from one to four copies of the X chromosome demonstrates the detection of single-copy deletions, monoallelic duplications (trisomies), and amplifications on Illumina's Human-1 (Figure 1A-1D) and HumanHap300 Genotyping BeadChips (Figure 1E)7. The changes in both the log R ratio and allelic ratios typically observed are listed in Table 1. A genomic DNA sample from a male was used to model a hemizygous deletion and a genomic DNA sample from a female was used as a control. Monoallelic duplication and amplification were modeled using XXX and XXXX cell lines, respectively. For a single SNP, the log R ratio response increased with increasing X copy number for the X chromosome but not for an autosome (Figure 1E). The log R ratio response was calculated for the X copy cell lines with a 10-SNP moving average for both array formats, which reduces the standard deviation (Figure 1F).

**Table 1: Detection of Copy Number Changes Using Infinium Whole-Genome Genotyping**

| Cell line | Aberration Modeled | Copy Number Change | Log R Ratio Change | Allelic Ratio(s) |
|---|---|---|---|---|
| XY (male) (Figure 1A) | Hemizygous Deletion | 2 to 1 | ~0 to -0.53 | Loss of Heterozygotes |
| XX (female) (Figure 1B) | Normal | No Change | No Change | 1:1 |
| XXX (Figure 1C) | Monoallelic Duplication | 2 to 3 | ~0 to +0.35 | 2:1 and 1:2 |
| XXXX (Figure 1D) | Amplification | 2 to 4 | ~0 to +0.54 | 3:1, 2:2, 1:3 |

**Figure 1: Detection of Changes in DNA Copy Number Using Human-1 and Humanhap300 Genotyping Beadchips**




Single copy deletions, monoallelic duplications, and amplifications are modeled on the Human-1 BeadChip (109K; A-D) and the HumanHap300 BeadChip (317K; E) using cell lines with one to four X chromosomes. All plots are shown juxtaposed with normal genomic profiles from chromosome 10 and were created with Illumina BeadStudio software.

(A) For XY, the presence of a single X chromosome is shown as a decrease in the log R ratio. In the Allele Frequency (AF) plot, the heterozygous state completely collapses to the homozygous axis.

(B) For XX, the presence of the expected two copies of the X chromosome shows no deflection in the log R ratio. In the heterozygotes are clustered around +0.5.

(C) For XXX, the log R ratio increases. The heterozygous state splits into two clusters representing a 2:1 and 1:2 ratio.

(D) For XXXX, the log R ratio increases even further. The heterozygous state is divided into three populations with allelic ratios of 3:1, 2:2, and 1:3, respectively.

(E) The response of the log R ratio for both the X chromosome and chromosome 10 for each X-copy number cell line on both the Human-1 and HumanHap300 Genotyping BeadChips. The log R ratio increases with increasing X-copy number of the X chromosome but not for chromosome 10. The corresponding standard deviation is shown. Note the similarities between Infinium I and Infinium II Assay-derived results. This data was generated with a 10-SNP moving average.

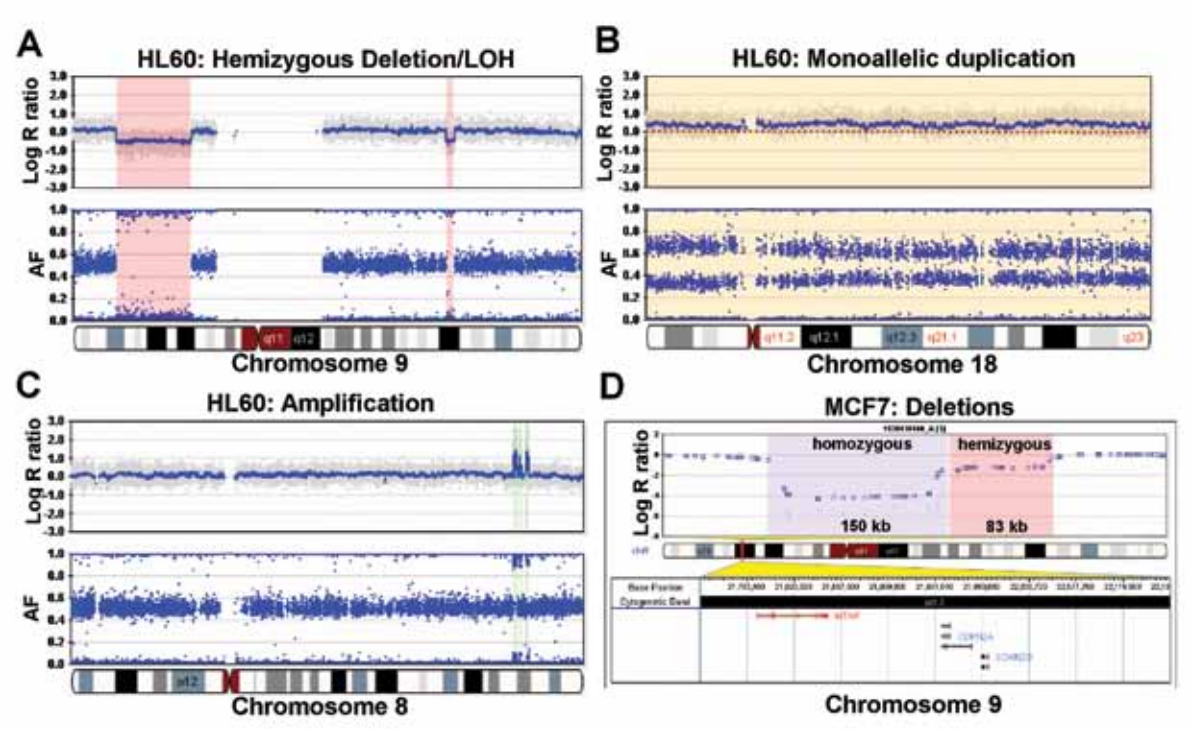
## Examples of Allelic Imbalance in Cell Lines

The ability of the HumanHap550 Genotyping BeadChip to detect allelic imbalances in the cancer cell lines HL60 (promyelocytic leukemia) and MCF7 (breast adenocarcinoma) is shown in Figure 2. All of the plots shown were generated with the Illumina Genome Viewer in BeadStudio. In HL60, deletions of varying size appear on Chromosome 9: a ~20Mb deletion on the p arm, and a ~1.8Mb deletion on the q arm (Figure 2A). These hemizygous deletions (from two copies to one copy) are manifest as a downward deflection in the log R ratio and a loss of heterozygotes in the AF. Other examples of aberrations in HL60 include monoallelic duplication of Chromosome 18 as indicated by an increase in the log R ratio and the split of the heterozygotes into two states: one at 0.67 (2:1 ratio) AF and another at 0.33 (1:2 ratio) AF (Figure 2B). There are also several small amplifications of approximately 260kb in HL60 on Chromosome 8 with 1:9 and 9:1 allelic ratios (Figure 2C). All of these aberrations have previously been identified using SKY karyotyping8. Adjacent homozygous and hemizygous deletions are revealed in MCF7 (Figure 2D). The gene annotation provided in the Illumina BeadStudio software allows users to quickly analyze affected gene regions. In this case, both copies of MTAP are homozygously deleted and CDKN2A (p16) and CDKN2B (p15) are hemizygously deleted.

## Summary

Illumina's Infinium Assays and Whole-Genome Genotyping BeadChips offer genome-wide coverage at an unprecedented resolution. The combined measurement of allelic ratios and normalized intensities provides enhanced detection of aberrations while facilitating identification of copy-neutral genetic anomalies such as uniparental disomy (UPD) and mitotic recombination9,10. Whole-Genome Genotyping can also yield allelic information on deletions, duplications, and amplifications, which have implications in cancer therapeutics. The combination of proven assays, high-density arrays, and integrated software enables the analysis of LOH and copy number changes in both single and paired (i.e., matched) samples with high precision.

**Figure 2: Detection of Allelic Imbalances in the Cancer Cell Lines HL60 and MCF7**




Genomic profiles of cancer cell lines on the HumanHap550 Genotyping BeadChip generated with Illumina BeadStudio software.
(A) Two regions in HL60 that exhibit LOH are shown by a downward deflection in the log R ratio and loss of heterozygotes in the AF profile.
(B) The entire length of Chromosome 18 in HL60 exhibits a monoallelic duplication (trisomy), as evidenced by the elevated log R ratio and split heterozygous cluster.
(C) Several amplifications in HL60 are present on Chromosome 8.
(D) High-resolution analysis of MCF7 showing a 150kb homozygous and 83kb hemizygous deletion in a region on Chromosome 9 containing MTAP, CDKN2A (p16), and CDKN2B (p15). For all log R ratio profiles, the blue line indicates a 100kb moving median for the HumanHap550 Genotyping BeadChip.

## References


1. The majority of the data described in this Application Note are published in the references listed in bold type.
2. **Steemers FJ, Chang W, Lee G, Barker DL, Shen R., et al. (2006) Whole-Genome Genotyping with the single-base extension assay. Nat Methods 3(1): 31-3.**
3. Whole-Genome Genotyping with the Sentrix® HumanHap550 Genotyping BeadChip and the Infinium™ II Assay. Illumina Technical Bulletin. Pub. No. 370-2006-017.
4. Albertson DG, Pinkel D (2003). Genomic microarrays in human genetic disease and cancer. Hum Mol Genet 12(Spec No 2): R145-52.
5. LaFramboise T, Weir BA, Zhao X, Beroukhim R, Li C, et al. (2005) Allele-specific amplification in cancer revealed by SNP array analysis. PLoS Comput Biol 1(6): e65
6. Schwab M. (1999) Oncogene amplification in solid tumors. Semin Cancer Biol 9(4): 319-25.
7. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, et al. (2003) Alpha-Synuclein locus triplication causes Parkinson's disease. Science 302(5646): 841.
8. **Peiffer DA, Le JM, Steemers FJ, Chang W, Jenniges T, et al. (2006) High-resolution genomic profiling of chromosomal aberrations using Infinium Whole-Genome Genotyping. Genome Res 16: 1136-1148.**
9. Visit http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi for more information.
10. Bruce, S., Leinonen, R., Lindgren, C.M., Kivinen, K., Dahlman-Wright, K., et al. (2005). Global analysis of uniparental disomy using high density genotyping arrays. J Med Genet 42(11): 847-51.
11. Teh, M.T., Blaydon, D., Chaplin, T., Foot, N.J., Skoulakis, S., et al. (2005) Genomewide single nucleotide polymorphism microarray mapping in basal cell carcinomas unveils uniparental disomy as a key somatic event. Cancer Res 65(19): 8597-603.


**Illumina, Inc.** • 9885 Towne Centre Drive, San Diego, CA 92121 USA • 1.800.809.4566 toll-free • 1.858.202.4566 tel • techsupport@illumina.com • illumina.com

FOR RESEARCH USE ONLY



illumina®