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Cytogenetic Analysis by High-Resolution SNP Array

Applications of the Infinium® CytoSNP-850K BeadChip Array.

Introduction

Array comparative genomic hybridization (arrayCGH) is commonly employed within cytogenetics to detect chromosomal abnormalities associated with developmental delay and/or congenital abnormalities. Virtually unheard of 10 years ago, it has now all but replaced karyotyping as the front-line screen for these applications in many cytogenetics laboratories world-wide.

Current array platforms incorporate a "backbone" of probes throughout the genome, with a larger number of probes clustered in regions of cytogenetic relevance. As the understanding of the human genome grows, it is becoming increasingly important to analyze a larger number of genes in greater detail. Precise information about the location of the breakpoints, and which genes are affected, is now essential for accurate interpretation. This information requires robust, high-resolution, intelligently designed arrays. In addition, the application of arrays is now widening to include more diverse scenarios, such as cancer and other technically challenging samples. The need for greater precision, as well as the broadening applications of arrayCGH, is therefore presenting new challenges for the cytogenetics laboratory.

The Infinium CytoSNP-850K BeadChip Array is a high-resolution, high-performance array that interrogates over 850,000 single nucleotide polymorphisms (SNPs) throughout the genome with

at least 15× redundancy. It provides both copy-number and loss of heterozygosity (LOH) data at high resolution. Intelligently designed with input from the international cytogenetics community, the array incorporates exon-centric enriched coverage of 3,262 genes derived from the International Collaboration for Clinical Genomics (ICCG; previously ISCA) and Cancer Cytogenomics Microarray Consortium (CCMC) cancer gene lists, as well as from Sanger gene lists, the Cancer Atlas, peer-reviewed publications, and external databases. The use of large 50-mer probes enhances the precision of the array, providing high signal-to-noise ratio and enhanced clarity of calls. SNP genotyping provides additional confirmation of the call and helps to delineate breakpoints further, as well as increasing sensitivity in the detection of mosaicism.

The Infinium CytoSNP-850K BeadChip Array integrates fully with BlueFuse® Multi software, making it a multifunctional tool for analyzing both constitutional and cancer samples. This dual functionality reflects the needs of the multi-functional cytogenetics laboratory—simplifying implementation and streamlining everyday workflow. This application note describes a proof-of-principle study that analyzed cytogenetics samples using the Infinium CytoSNP-850K BeadChip Array.

Figure 1: Hemizygous Deletion

A 164 kb hemizygous deletion including the *UBE2A* gene. The log R chart shows that no copies of this region were detected, while the B-allele frequency is plotted as scattered points, indicating that the SNP genotype could not be calculated. Data courtesy MGZ Medical Genetics Center, Munich, Germany.

Methods and Results

Performance of the CytoSNP-850K Array

To demonstrate the performance of the CytoSNP-850K array, and to enable users to compare it to their current arrayCGH platform, laboratories were invited to submit up to eight DNA samples as part of a proof-of-principle study.

Samples included those derived from blood, cell lines, bone marrow, amniocytes, and saliva. Laboratories were encouraged to submit cases such as uniparental disomy, mosaics of less than 20%, or those with unclear calls from a previous analysis. Arrays were processed, and results reviewed with the submitting laboratory.

Detection of Small Abnormalities

The CytoSNP-850K array incorporates a high density of probes within cytogenetically relevant genes. Over 96% of targeted genes contain at least 10 probes, with a mean of 59 probes per gene, resulting in an exon-centric design that provides the highest levels of targeted content. As a result, the array is able to detect clinically significant abnormalities as small as 1 kb (theoretical detection based on genes with the highest probe density). The 15× redundancy of probes and use of SNP genotyping data facilitates high confidence even in these small abnormalities, enabling clear differentiation between true calls and artifacts.

For example, Figure 1 shows a high density of probes in the *UBE2A* gene, allowing a hemizygous deletion that includes this gene to be detected. This same abnormality was missed when using an alternative array platform.
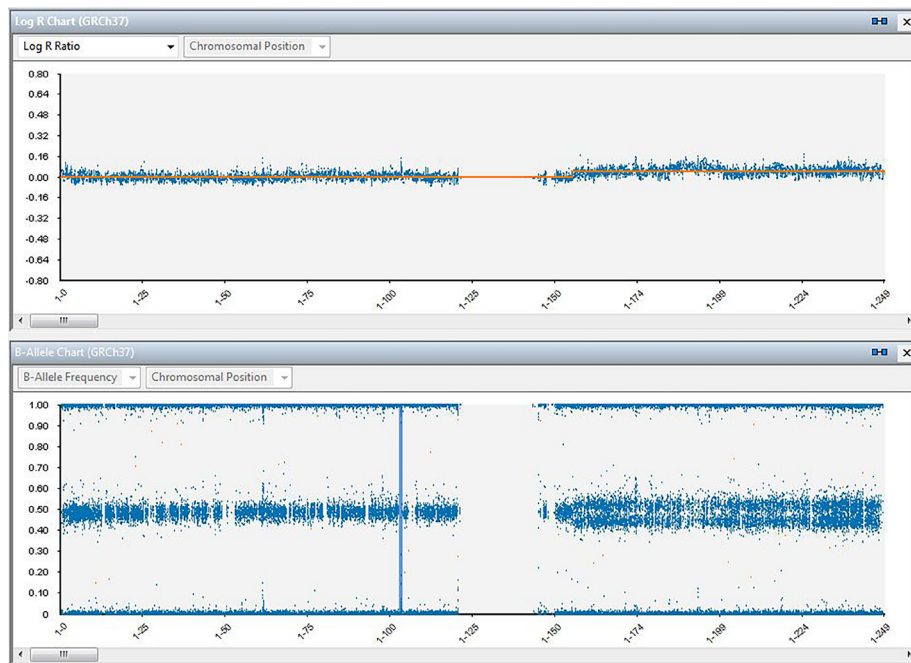
Detection of Mosaicism

The use of SNP probes, as well as advanced algorithms provided through BlueFuse Multi software, facilitates the detection of low-level mosaicism. Figure 2 shows a mosaic gain of most of the long arm of chromosome 1, which was detected using the CytoSNP-850K array, and can be clearly seen in the B-allele frequency chart.

Triploidy

Non-SNP arrays are restricted to analyzing only copy number, which makes it impossible to detect polyploidy, because each genomic region is quantified by comparison with other regions. The CytoSNP-850K array enables detection of these changes, as the allele ratios show distinct patterns throughout the genome. Figure 3 shows a case of triploidy. As expected, there is no change in log R, but the B-allele frequency chart clearly shows that the clustering of heterozygous SNPs has split into two distinct clusters reflecting 1:2 and 2:1 allele ratios.

Figure 2: Mosaicism

Mosaic gain of the long arm of chromosome 1. A low-level gain of 1q is clearly visible in the B-allele frequency. The heterozygous allele cluster has split into two clusters, reflecting allele proportions deviating slightly from 1:1. Data courtesy Amanda Dixon-McIver, IGENZ, Auckland, New Zealand.

Figure 3: Triploidy

No change in the log R is detected, but the B-allele frequency chart shows the characteristic 1:2 and 2:1 allele ratios across all chromosomes. Data courtesy Sullivan Nicolaides Pathology, Brisbane, Australia.

Uniparental Isodisomy
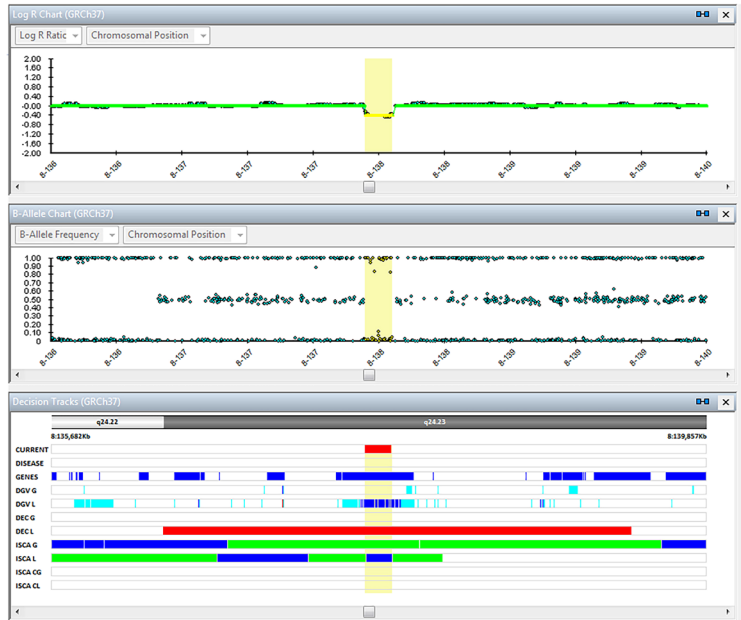
Uniparental isodisomy (UPD) is not detectable without the use of SNP probes as there is no change in copy number. The CytoSNP-850K array, however, enables the detection of UPD by identifying LOH across the whole chromosome. For example, Figure 4 shows a case of UPD of chromosome 15: no copy-number change is present, but LOH for the whole of chromosome 15 is clearly visible in the B-allele frequency chart.

Long Contiguous Stretches of Homozygosity (LCSH)

Detection of LCSH through the use of SNP probes can identify regions that are identical by descent and narrow the list of genes involved in a suspected recessive disorder. Any disease-causing sequence variants in these regions are likely to have been inherited on both copies of the chromosome and will therefore be homozygous.

Precision and Comparison with Other Platforms

Comparison of detected variants with known common CNVs verifies the precision and reliability of the CytoSNP-850K array. As seen in Figure 5, a detected loss within 8q24 lines up exactly with entries in the Database of Genomic Variants (DGV) and ISCA. This comparison not only provides confidence in the precision of calls, but aids interpretation by easily classifying CNVs.

Conclusions

During a proof-of-principle study, the CytoSNP-850K array was used to test a wide variety of samples from cytogenetics laboratories globally and to compare performance with other array platforms. This study demonstrated the advanced utility of the CytoSNP-850K array in detecting small abnormalities at high resolution, and it showed superior sensitivity in the detection of mosaicism. The array was also able to detect UPD, LOH, triploidy, and a number of other complex scenarios. Abnormalities were detected with precision, and SNP data added to the analytical utility of the array. This study verifies the applicability of the CytoSNP-850K array for cytogenetic analysis and confirms its status as a multi-functional and high-performance array.

Figure 4: Uniparental Isodisomy

UPD of chromosome 15. Data courtesy West Midlands Regional Genetics Laboratory, Birmingham, UK.a

Figure 5: Platform Comparison

A small 174 kb CNV, shown to line up precisely with copy-number losses reported from numerous other sources and listed on databases including DGV and ISCA. Data courtesy West Midlands Regional Genetics Laboratory, Birmingham, UK.

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