Insights & Perspectives

Applications of Whole-Genome Genotyping for the Profiling of Cancer Samples

An Interview with Illumina Post-Doctoral Scientist Daniel Peiffer

Daniel Peiffer played a central role in establishing the utility of the Infinium™ Assay for LOH and copy number analysis and was a key contributor to the development of the BeadStudio LOH Plus module. Dr. Peiffer works in Illumina's Genotyping group under the direction of Dr. Kevin Gunderson.

What key feature does Illumina provide to support cancer research and discovery?
Traditionally, array-CGH has been used as a major technique for discovering novel cancer phenomena. With Illumina's SNP-CGH, we have the ability to simultaneously obtain both intensity information (used for copy number analysis) and genotyping information (to detect allelic imbalances) using the same platform. Array-CGH does not provide any genotyping data. In the past, LOH analysis has been used in the discovery of several tumor suppressor genes, including p53 and Rb1. Now such analyses can be performed more rapidly; the time from assay initiation to analysis of variations in genomic structure is extremely short. HumanHap300 and HumanHap550 BeadChips, in conjunction with streamlined analysis using BeadStudio LOH Plus software, makes this an ideal platform for discovery-based cancer research.

How does Illumina's technology enable the detection of genetic aberrations that are considered a challenge to detect, such as a copy-neutral events?
Classical array-CGH (such as BAC [bacterial artificial chromosome] array-CGH) analysis of chromosomal aberrations is based on examination of normalized intensities between a reference and subject sample (paired samples). In contrast, SNP-CGH utilizes a combination of two genotyping parameters: a normalized intensity measurement and an allelic ratio. These parameters provide a more sensitive and precise profile of chromosomal aberrations than past techniques. Overall, SNP arrays have the power to detect common aberrations such as heterozygous deletions (LOH), homozygous deletions, and amplifications. In addition, SNP arrays can detect copy-neutral LOH events (LOH with no apparent copy number change), which cannot be detected using array-CGH.

Several recent studies have described copy-neutral aberrations in tumors. The ability to detect these events is becoming extremely important for tumor analysis and classification.

How does Illumina's BeadStudio software help scientists visualize their data?
The BeadStudio LOH Plus module was developed to make visualization of SNP-CGH data nearly effortless and more applicable to a clinical setting. Tools in the LOH Plus module allow users to intuitively view chromosomal aberrations on a genome-wide scale using the Genome Viewer, cluster various data parameters along the length of individual chromosomes to identify trends using the Chromosome HeatMap, and annotate regions of interest both manually and automatically (Bookmarking, Autobookmarking). In addition, with the addition of the LOH Plus module, it is

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now possible to analyze paired samples, such as a matched normal/tumor pairs (as opposed to single-sample analysis, which uses the cluster file as a reference). This drastically increases the discovery power for LOH and copy number analyses.

How do researchers evaluate the very large data sets often needed to study polymorphisms and genetic defects that may lead to cancer?

There is definitely a bottleneck in analyzing such large data sets. After gathering such a large amount of data from hundreds or even thousands of samples, it can be difficult to know where to start mining the data! To evaluate such large sample sets for various types of chromosomal aberrations, a researcher can use various tools in BeadStudio. For example, we have an algorithm that calculates LOH scores and copy number changes for all samples. These scores can be clustered along the length of the genome so that researchers can identify and analyze regions with common variations.

There must exist instances where a so called "needle in a haystack" genetic event occurs. How do scientists uncover such instances?

As the feature density on Illumina's BeadChips increases, the effective resolution for discovering aberrations such as microdeletions is better than ever before. For the Infinium Whole-Genome Genotyping Assays, the HumanHap-300 has a median SNP spacing of 5.4kb, enabling an effective resolution of approximately 54kb. The HumanHap550 narrows the resolution to approximately 28kb. Higher density increases the sensitivity in analyzing tumor samples contaminated with normal tissue, which turns out to be the case for almost all cancer samples. We have worked with cytogenetics laboratories to identify novel aberrations of approximately 70kb in samples that have been studied many years with other traditional platforms.

In terms of finding the "needle in a haystack" after obtaining data from hundreds of samples, researchers can mine large data sets for trends and commonalities using the tools supplied in BeadStudio. Then, they can use the Chromosome Browser to further analyze high-resolution SNP-CGH data. With this tool, it is possible to focus in on just a few SNPs to analyze their performance in a sample of interest. In this way, it is possible to determine the exact breakpoints of a chromosomal deletion, for example, which is of paramount importance so genes that span this region can be identified.

Further Reading:

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ADDITIONAL INFORMATION

To learn more about the BeadStudio2.0 LOH Plus Module, visit our website or contact us.

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