UHNMAC News

The University Health Network Microarray Centre Newsletter – Winter 2009

UHN Microarray Centre

Affymetrix Authorized Service Provider

Agilent Certified Services Provider Microarray-Based Genomic Analysis

Happy New Year!

This edition of the UHNMAC News features a review of a recent publication that uses UHNMAC HCGI12K arrays to identify a gene that may modify the metastatic risk of uveal melanoma.

Ever wondered if next-generation sequencing (NGS) and microarrays are complimentary or competing technologies when it comes to transcriptome profiling and DNA-protein interaction studies? Check out the feature report to learn more. To link to an article about NGS and microarrays, published by GenomeWeb BioArray News, click here.

Announcements

Novel gene identified

Carl Virtanen, James Paris, and Mark Takahashi (members of the UHNMAC) have recently identified and characterised a novel gene, Differentiation Associated Protein (DAPR) (J Biol Chem, 2008, Epub ahead of print)

A review of this paper is available on our website.

Agilent scanner upgrade

The Agilent Scanner will be upgraded in January 2009 to enable 2 µm scanning resolution. Check our website for more updates.

Feature Article & Review

Looking into eye cancer: The identification of a gene that may modify metastatic risk.

Review of: Onken MD, Worley LA, Harbour JW. A Metastasis Modifier Locus on Human Chromosome 8p in Uveal Melanoma Identified by Integrative Genomic Analysis. Clin Cancer Res 2008, 14(12):3737

As metastasis is the leading cause of death in cancer patients, identifying therapeutic targets that interfere with the metastatic process is critical (1). Many oncogenes and tumour suppressor genes have been identified, but only about 10 metastasis suppressor genes have been identified

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Feature Report

Next-generation sequencing: Synergy with Microarrays

Next-generation sequencing (NGS), also called massively parallel sequencing, has been commercially available since 2004. Unlike dye-terminator sequencing using capillary electrophoresis, which can process 96 samples at a time, NGS increases sequencing throughput by laying millions of DNA

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Uveal melanoma

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(2). Uveal melanoma is a common intraocular malignancy that results in aggressive metastases in about half of all cases (3). This particular cancer is valuable for studying metastasis as it has a well characterised gene expression signature associated with metastasis and the metastasis occurs mostly by the hematogenous route rather than by local invasion or lymphatic dissemination (1,3). The two distinct expression signatures of uveal melanoma have already been described (4). Tumours with the class 1 signature have a very low risk of metastasis, whereas those with the class 2 signature have a high risk of metastasis. In this study, the class 2 tumours were further segregated into two subgroups, class 2A and class 2B (1). Class 2B tumours were associated with more rapid onset of metastasis than class 2A tumours.

Onken and colleagues have previously reported that the development of the class 2 gene expression signature is usually accompanied by the deletion of chromosome 3, suggesting that one or more genes on chromosome 3 may be associated with metastatic progression (5). The purpose of this current study was to identify genes that modify metastatic risk in uveal melanoma. To this end, researchers analysed 53 primary uveal melanomas by various integrative genomic methods.

Array CGH and SNP-based detection of loss of heterozygosity

Using array CGH, chromosomal copy number changes were assessed in 19 of the class 2 tumours. Of the various copy number changes identified, the only alteration that correlated with time to metastasis was 8p loss. SNPs across chromosome 8p were interrogated for loss of heterozygosity to further define the minimal deleted region, which consisted of a 10Mb stretch from 8p22 to 8p12. Leucine zipper tumour suppressor-1 (LZTS1), one of the 11 genes located within the deleted region, was most strongly linked to rapid metastasis.

Global DNA methylation profiling

In addition to the deletion of chromosome 8p12-22, DNA hypermethylation of the corresponding region of the retained hemizygous 8p allele was associated with more rapid metastasis. DNA from six tumours was interrogated using methyl-DNA binding columns followed by hybridisation of the enriched samples to UHNMAC HCGI12K arrays. Global DNA methylation profiling showed that the LZTS1 promoter was hypermethylated and silenced in rapidly metastasising tumour cells and not in slowly metastasising or non-metastasising cells.

Uveal melanoma cells overexpressing LZTS1 exhibited decreased invasion and motility while cells depleted of LZTS1 ... exhibited increased motility.

Gene expression profiling

The expression of LZTS1 was further evaluated. Uveal melanoma cells overexpressing LZTS1 exhibited decreased invasion and motility while cells depleted of LZTS1 using small interfering RNA exhibited increased motility. The proliferation rate in uveal melanoma cells was not altered by modulation of LZTS1 mRNA levels. Other studies have reported that LZTS1 functions, in part, as a mitotic regulator (6) and can inhibit cancer cell growth through regulation of mitosis (7).

Using various integrative genomic methods, Onken and colleagues have shown how genetic modifiers of metastatic risk can be identified in human cancers. This study reveals that the silencing of a region on chromosome 8p modifies the metastatic efficiency of class 2 primary uveal melanomas.

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Next-generation sequencing

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fragments on a single chip and sequencing all fragments in parallel (1). DNA fragments, which can be collected from a number of upstream processes, are used to build DNA fragment libraries that are subsequently used as sequencing templates. DNA fragment libraries are prepared for sequencing by ligating specific adaptor oligonucleotides to both ends of each fragment (1,2). Following sequencing, informatics allows each sequencing read to be mapped to a reference genome. The goal of this report is to describe the impact NGS will have on microarrays and provide a summary of three popular NGS platforms.

NGS platforms can be used for transcriptome profiling, miRNA profiling, DNA-protein interaction studies using chromatin immunoprecipitation (ChIP), and DNA methylation studies, thus challenging the use of microarrays. However, several studies have concluded that microarrays and NGS are actually complementary platforms, rather than competitive alternatives, that should be used together to gain the maximum results (3-5). Euskirchen *et al*. compared ChIP-chip with ChIP-sequencing and found that each method detected targets that were missed by the other method (3). For transcriptome profiling, Oudes compared Affymetrix GeneChip Array data with Massively Parallel Signature

Table 1. The strengths and weaknesses of NGS and microarrays.
(This table was taken from Asmann *et al*. Gastroenterology 2008, 135:1466)

Microarray Analysis		Massively parallel, or next-generation, sequencing	
Pros	*Cons*	*Pros*	*Cons*
Relatively inexpensive	High background, low sensitivity	Low background, very sensitive	Expensive
Easy sample preparation	Limited dynamic range	Large dynamic range	Complex sample preparation
Mature informatics and statistics	Not quantitative	Quantitative	Limited bioinformatics
	Competitive hybridisation		Massive information technology infrastructure required
	Annotation of probes		

Sequencing (MPSS) and found that each method detected genes that the other did not and concludes that transcriptome profiling with a single methodology will not fully assess the expression of all genes (4). Researchers considering replacing microarrays with sequencing platforms should be aware of the advantages and disadvantages associated with both methods (Table 1). NGS has some advantages that cannot be rivaled by current microarray platforms, including lower background, better sensitivity, and quantitative measures (6). However, a sophisticated information technology infrastructure is necessary to manage the vast amount of sequence data (6,7), and the future success of NGS will depend on the development of new algorithms to perform alignments and handle large amounts of data efficiently (7).

NGS platforms

Three commercially available NGS platforms, including Roche (454) FLX Genome Sequencer, Illumina Genome Analyzer II, and Applied Biosystems' SOLiD™ (Sequencing by Oligo Ligation and Detection) Sequencer, will be briefly described. Table 2 compares the specifications of each platform. The information presented in this report was collected in November 2008 (8-10) and was intended to provide a general comparison. We highly recommend that you contact each manufacturer for current performance specifications.

It should also be mentioned that single molecule sequencers, so-called "third-generation sequencers", have been developed. Helicos' HeliScope, which employs True Single Molecule Sequencing (tSMS) technology to sequence samples without amplification, is capable of producing over 10 Gb of sequence data per 8 day run (11). Pacific Biosciences has developed Single Molecule Real Time (SMRT™) sequencing technology which involves proprietary surface and nucleotide chemistries (12). This sequencer, which promises longer reads, shorter run times, and higher quality data, is expected to be released in 2010 (12).

Sequencing chemistry

Pyrosequencing (Roche platform) involves the use of a pyrophosphate molecule, released following nucleotide incorporation by DNA polymerase, to propagate reactions that ultimately produce light. Illumina's sequencing-by-synthesis involves the use of four differently labelled fluorescent nucleotides that have their 3'-OH groups chemically inactivated to ensure only a single base is incorporated per cycle. Each base incorporation cycle is followed by an imaging step to identify the base incorporated, and a chemical step that removes the fluorescent group and deblocks the 3' end for the next base incorporation cycle. The SOLiD sequencer (Applied Biosystems) uses a ligation-based sequencing process that starts by annealing a universal sequencing primer that is complementary to the SOLiD-

Table 2. Comparison of Roche, Illumina, and Applied Biosystems NGS platforms.

	Roche (454): Titanium series reagents (run on FLX Genome Sequencner)	Illumina: Genome Analyzer II	Applied Biosystems: SOLiD™
Sequencing chemistry	pyrosequencing	polymerase-based sequencing-by-synthesis	Ligation-based sequencing
Amplification approach	Emulsion PCR	Bridge amplification	Emulsion PCR
MB/run	400-600 MB	1300 Mb	3000 MB
Time/run	10 hr	4 days	5 days
Read length	400 bp	up to 75 bp	35 bp

specific adaptors on the library fragments. Then, a limited set of semi-degenerate 8-mer oligonucleotides (and DNA ligase) is added. When matching 8-mers hybridise to DNA fragment sequences adjacent to the universal primer, DNA ligase seals the phosphate backbone and a fluorescent readout identifies the fixed base of the 8-mer. A subsequent cleavage step removes bases 6-8 of the ligated 8-mer, removing the fluorescent group and enabling another round of 8-mer ligation, and so on. The advantage of ligation-based sequencing chemistry is the "built-in" quality check of read accuracy (2).

Amplification approach

Emulsion PCR is the amplification approach used by both Roche and Applied Biosystems. For the Roche platform, emulsion PCR is carried out on the DNA fragments attached to the surfaces of agarose beads. On the Applied Biosystems sequencer, the DNA fragements are attached to the surfaces of magnetic beads. Bridge amplification (Illumina) is a PCR reaction that occurs within a discrete area of the flow cell surface.

Read length/Mb per run/Time per run/Approximate cost

NGS produce shorter reads (25-400 bp) than capillary sequencers (650-800 bp), but the read length varies considerably among NGS. Roche provides the longest read (400bp with the Titanium system) while Illumina and Applied Biosystems are much shorter (32-75 bp and 35 bp, respectively). The advantageous read length and run time of the Roche platform is offset by its cost (the Roche GS FLX sequencer's "cost-per-run" is more expensive than the other two; 2) and the lower run yield (roughly between 20% and 46% of the sequence data that could be obtained by using the Applied Biosystems and Illumina systems).

Be sure to read "Cost, Data Analysis, and Throughput Keep Array Users from Switching to DGE, Some Say" by Justin Petrone, December 23, 2008, on GenomeWeb BioArray News. This article discusses digital gene expression (DGE) performed on second-generation sequencing platforms and the impact DGE could have on microarrays.

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