

October 2011



## AROS Applied Biotechnology

QUARTERLY NEWSLETTER

October- 2011

### Fluidigm offers high quality PCR-based gene expression analysis from single cells at attractive prices

*The majority of gene expression studies have been conducted on seemingly identical cell populations. However, variations in gene expression may differ significantly on a cell-to-cell level. Single cell analysis provides new knowledge on gene expression patterns in cells. Single cell expression profiling has wide applications especially in the field of prenatal diagnostics, but also in the investigation of immunological, neurological, oncological and developmental problems.*

With 10–30 pg of total RNA in a typical mammalian cell, and with only between 1–5% of this being mRNA, there are clear limitations of using conventional microtiter plates for single cell profiling. The challenge is further underpinned by the fact that some highly expressed genes account for up to 3% of the transcripts, whereas low abundance transcripts have a copy number of only 5–15 molecules per cell.

At AROS we have implemented the Fluidigm BioMark System as one of the main tools for qPCR-based expression analysis. The Fluidigm 96.96 Dynamic Array IFC enables the analysis of 96 samples versus 96 individual transcripts in one experiment, with the time consumption of only three hours, and the sensitivity of the BioMark System being comparable to conventional qPCR Systems.

The Fluidigm setup is ideal for running qPCR experiments on single cells. It is possible to run 96 experiments using the contents of a single cell, as well as running several plates; the literature even describes running up to 1,000 experiments from one cell using standard TaqMan® and DNA binding dye assays. The protocol "Fluidigm Dynamic Arrays for Single-Cell Gene Expression Analysis", which is used at AROS for single-cell gene expression analysis is a technique that produces inexpensive and reproducible gene expression results from single-cell samples. With this protocol it is possible to determine single-gene cell expression levels in everything, e.g. circulating tumor cells (CTCs), human cells from eight-cell-stage embryos and stem cells.

FACS products are sorted directly into 5µl RT/PCR master mix solution. After lysis and reverse transcription, the resulting cDNA is specifically target amplified with pools containing up to 96 TaqMan probes. The amplified cDNA is loaded onto a Fluidigm Dynamic Array with up to 96 genes, generating 96 amplification data points per cell, and a total of 9,216 data points per experiment.

This procedure allows a large number of cells and genes to be tested at the same time, with data quality rivalling benchmark real-time qPCR results.



### New miRNA service

Over the last 3 years, AROS has built a comprehensive portfolio of competences with respect to miRNA analysis, including in-house developed extraction protocols as well as numerous microarray and PCR-based miRNA expression methodologies. Exiqon has now appointed AROS a Center of Excellence for providing real-time PCR services based on Exiqon's miRCURY LNA™ Universal RT microRNA PCR product line, which is now added to our portfolio of miRNA offerings.

LNA™-based microRNA profiling holds great promise as a non-invasive way to discover important new biomarkers for a wide range of diseases and biological processes. The extreme sensitivity obtained by inclusion of LNA™ in the PCR primers enables the use of non-invasive samples like serum and plasma, where the quantity of RNA is extremely low.

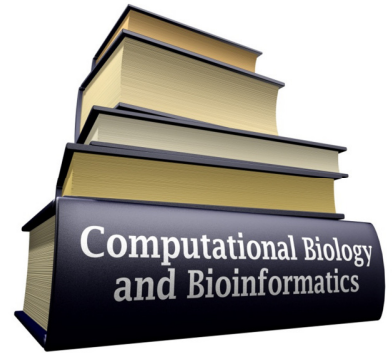
## AROS now also offers bioinformatics service

**Combined outsourcing of laboratory analyses and bioinformatics is the obvious choice. For many customers, the often daunting task of bioinformatics analysis of the vast amounts of data is overwhelming, mainly because it is so highly time consuming and specialized to be conducted in house.**

AROS has occasionally assisted in smaller bioinformatics tasks in the past. In order to meet the increasing demand in bioinformatics assistance, AROS now offers a larger variety of bioinformatics services at competitive prices. These services include the analysis of Affymetrix and Illumina based gene expression analyses across samples, and SNP profiling for Mendelian and population genetics. Also, analysis of NGS data produced on our Illumina HiSeq platform is provided, allowing for genome, exome and transcriptome sequence assembly, InDel and SNP variant detection, as well as the determination of gene expression variation.

Amongst others, the new services include: **Filtering and normalization, Mapping and assembly of reads, Gene finding and annotation, Alignments to reference genomes, Variant calling** (SNPs, Indels, CNVs, splice variants), **Assessment of significance of variance across samples, Cross-referencing with existing genomic resources** (SNPdb, HapMap, 1000Genomes) and **Custom analyses** such as Clustering, Principal Component Analysis, and building classifiers.

The pricing depends on the volume of the analysis requested, making this service highly flexible. Upon request, GxP specifications can be followed.



### AROS has run the first sample sets on the Affymetrix CytoScan HD

**With their new CytoScan HD, Affymetrix has launched a new high-density platform for cytogenetics research to replace their Whole Genome 2.7M array.**

With 2.6 million markers, the new chip offers, according to Affymetrix, a 100 percent coverage of cancer genes as well as X chromosome genes (because 25 percent of known syndromes are on the X chromosome); the array also offers 100 percent coverage of constitutional genes.

According to Affymetrix, the array provides coverage of not only clinically relevant genes today, but clinically relevant genes tomorrow, meaning every single gene in the genome is covered.

750,000 of the markers on CytoScan HD are SNPs. The SNP content in the CytoScan HD will enable researchers to detect uniparental disomy, loss-of-heterozygosity, and consanguinity, conditions that comparative genomic hybridization arrays have been incapable of detecting.

### Optimization of exome-sequencing at AROS

**The exome of a genome is formed by all the protein-coding sequences, and is therefore likely to contribute to the phenotype of an organism. This, in combination with its small size (the human exome is estimated to comprise only 1.5 % of the genome) makes the exome a very interesting sequencing target.**

At AROS, exome targeting, enrichment and sequencing is accomplished with Illumina's TruSeq Exome Enrichment Kit in combination with HiSeq2000 sequencing. The enriched 62 Mbase region covers 20,794 genes, including 5' and 3' UTRs, as well as microRNAs and other non-coding RNAs. The protocol allows for pooling of up to 6 genomic DNA libraries before exome enrichment, and whilst this reduces cost and hands-on time for large scale studies, it also adds a critical step to the protocol: precise normalization of the concentration of the individual DNA libraries in the pool.

At AROS we have implemented a series of steps to ensure the right concentrations of the individual DNA libraries. The different steps are integrated in AROS's workflow for quantification of all final sequencing libraries before flow cell loading and cluster amplification on the cBot. This ensures the correct estimation of the library molecules that can actually form clusters in the downstream bridge-PCR process of cluster amplification. Importantly, the inclusion of a normalization control at this point allows for an extremely efficient use of the HiSeq2000 sequencing capacity. With a view to maximize the sequencing output and, as a minimum, meet Illumina's specifications for every HiSeq2000 run, AROS will continue to optimize the workflow.

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