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Complimenting two generations of DNA Sequencing

Inauguration of next generation

Applications offered by Illumina Genome Analyzer

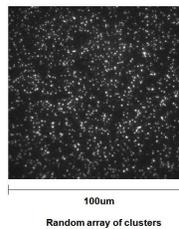
- Genome DNA sequencing using single or paired-end reads
- Discover and confirm SNPs
 - Identify chromosomal rearrangements, including Copy Number Variations (CNVs)
 - Map break points
 - Detect rare variants

Digital gene expression

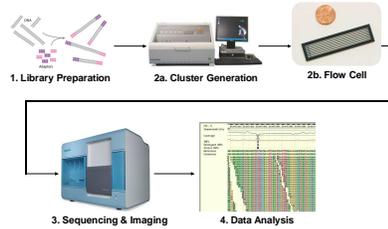
- novel RNA discovery
- accurate quantification of low abundance RNA
- orthogonal microarray validation

ChIP-Seq supports genome-wide study

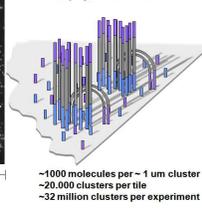
- Transcription factors
- Polymerases and transcriptional machinery
- Structural proteins such as histones and histone variants
- Protein modifications such as methylated histones and phosphorylated proteins



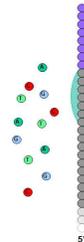
Workflow in Illumina Solexa Genome Analysis System



Attach single molecules to surface
Amplify to form clusters

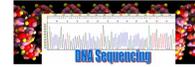


~1000 molecules per ~1 um cluster
~20,000 clusters per tile
~32 million clusters per experiment



- Cycle 1: Add sequencing reagents
First base incorporated
Remove unincorporated bases
Detect signal
- Cycle 2-n: Add sequencing reagents and repeat
- All four labelled nucleotides in one reaction
 - High accuracy
 - Base-by-base sequencing
 - No problems with homopolymer repeats

Conventional Sanger DNA Sequencing



- Plasmid DNA
- PCR amplicons
- BAC/PAC/Cosmid DNA
- Gene walking



- Current instrument—ABI 3730 DNA Analyzer, 48 capillary array
- Sanger DNA sequencing with BigDye 3.1 terminator chemistry
- Average read length—750 bp
- Capacity—1.2 Mbp/day
- DNA Fragment Analysis
- Microsatellite Genotyping (STR)
- SNP Genotyping
- ALFP Analysis

Microarrays on Various Platforms

Background

Gene Expression Analysis. Our vision is that Affymetrix GeneChip analysis is the best option for monitoring global mRNA expression. Various small-scale arrays (made in house and commercial) are available as a choice for investigation of specific groups of genes with multiple experimental variables.

Protein arrays. The protein microarrays are an emerging class of proteomic technologies, which are fast becoming critical tools in molecular biology. Two formats of protein microarrays are currently available: forward phase arrays (FPA) and reverse phase arrays (RPA). In FPAs, capture molecules, usually an antibody, are immobilized on the support and act as a bait molecule. Each spot contains one type of immobilized antibody specific for one protein. In the FPA format, each array is incubated with one sample and multiple analytes are measured at once. In contrast, the RPA format immobilizes an individual test sample in each array spot. Such array is comprised of hundreds of different patient samples or cellular lysates and a single analyte endpoint is measured and directly compared across multiple samples.

DNA arrays for miRNA analysis. MicroRNAs are non-protein coding RNA genes that reside within longer transcripts as distinct hairpins and mature into 22 base RNA sequence-specific gene regulators. miRNAs are believed to mediate posttranscriptional gene repression. Recent studies link miRNA expression to cancer progression, viral infections, and brain development.

Microarray Data Analysis and Data Mining and Bioinformatics. The DNA Sciences Core works closely with Academic Computing for Health Sciences (ACHS) and the Microarray Bioinformatics Core to assure the most efficient usage of the microarray data. Within the DNA Sciences Core, a great deal of resources has also been devoted to mining the data in an increasingly new depth. The Core has licensed subscription to the highly acclaimed Ingenuity knowledge database and through it to furnish in depth annotation of all the microarray data.

Microarray services

- All steps of microarray analysis starting from cell or tissues.
- All standard Affymetrix GeneChip analysis.
- Any types of commercially available slide arrays (DNA or protein).
- Integrated projects: microarray analysis and qPCR validation of the genes found regulated by microarray analysis.

Formats

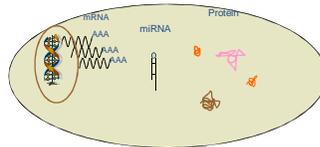
DNA Microarrays

- Affymetrix GeneChip
- Spotted arrays
- Plate arrays

Protein Microarrays

- Forward phase arrays
- Reverse phase arrays

Applications



DNA Microarrays

- Genomic analysis
- SNP
- Transcriptom
- DNA Sequencing

Protein Microarrays

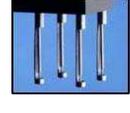
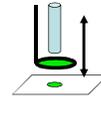
- Gene expression
- mRNA
- miRNA
- Protein
- Functional analysis
- Antigen-antibody
- Protein-Protein
- Ligand-receptor
- Enzyme-substrate

Instrumentation

Affymetrix GeneChip System



Microarray spotting robot



- Confocal scanning
- Three internal lasers
- 5 micron pixel resolution

PE ProScanArray Scanner



Real Time Quantitative/RT PCR

Applications

- Gene expression Quantification
- SNP Characterization and Screening
- microRNA Quantification
- DNA Copy Number Determination
- Viral Titration

The Group



ABI 7900 HT DNA Detection System
Barcode reading Capacity
Fast Block to adopt
Assay design and validation

Joint Venture with Commercial Companies



Oligosynthesis can be ordered from both in-house or through the UVA-Invitrogen SupplyCenter program, both complementary to each other.

PCR arrays can be ordered via a soon coming UVA-supperArray program. The special application for this array type is to validate the pathway discovery in microarray experiments.