

Microarray technology

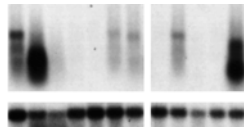
Leonardo A. Meza-Zepeda
 Microarray Core Facility
 Oslo University Hospital Rikshospitalet/University of Oslo
 Norwegian Microarray Consortium
 running the
 FUGE microarray platform
 oslo@microarray.no



Measuring gene activity

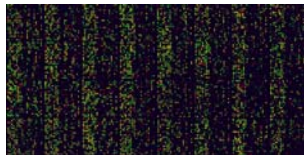
Molecular biology

- One gene analysed in many samples on blots
- The samples are immobilised



Functional genomics

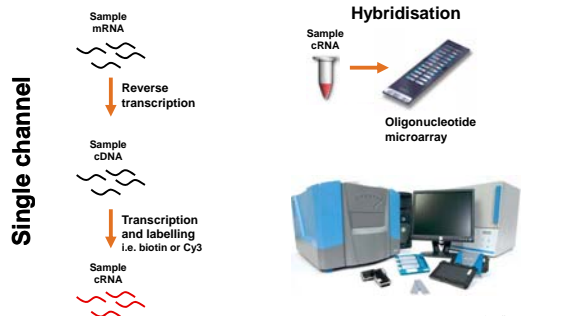
- Thousands of genes analysed in parallel
- The probes are immobilised



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Microarray experiment

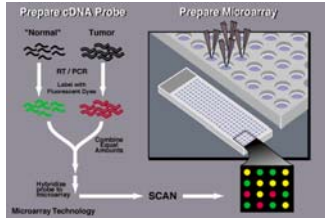


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Two channels

- Two samples per array
- Competitive hybridization
- Uneven degradation of Cy-dyes
- May need dye-swaps (doubles the cost)



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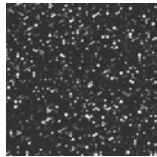


Microarray experiment

Laser scanning



Image acquisition



Signal intensity

Amount of mRNA

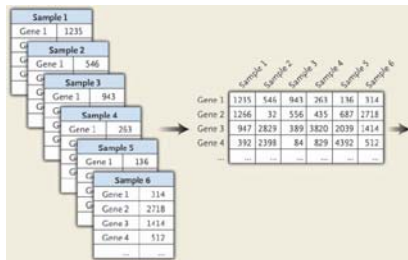


(http://www.illumina.com)

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Dataset



- Hybridization Quality Control
- Data extraction
- Exporting datasets

(Quackenbush, NEJM, 2009)

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Types of arrays

cDNA arrays (cloned "mRNA fragments")

- Expression analysis
- Gene copy number analysis

Oligonucleotide arrays (synthetic DNA)

- Sequence (mutation) analysis
- Expression analysis
- Gene copy number
- Epigenetics

Genomic arrays (fragments of chromosomes)

- Gene copy number analysis
- Epigenetics

Protein arrays (antigens, antibodies, aptamers, etc.)

- Protein interactions (also with DNA, small molecules etc.)

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Commercial platforms

Platform	Technology	Organism
Affymetrix	<ul style="list-style-type: none">• Synthesized probes• One channel (biotin)	<ul style="list-style-type: none">• Several
Agilent	<ul style="list-style-type: none">• Spotted on glass slides• One/Two channel (Fluorescence)	<ul style="list-style-type: none">• Several
Illumina	<ul style="list-style-type: none">• Beads• One channel (biotin)	<ul style="list-style-type: none">• Human• Mouse• Rat
Nimblegen	<ul style="list-style-type: none">• Synthesized• One channel (Fluorescence)	<ul style="list-style-type: none">• Several

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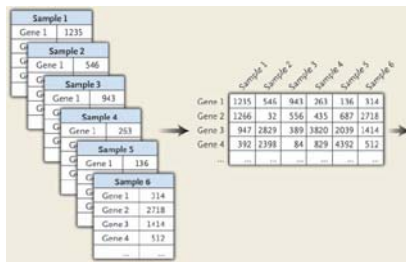
Which platform to use?

- Organism
- Budget
- Amount of totalRNA
- Number of samples
- Flexibility of the platform

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Dataset



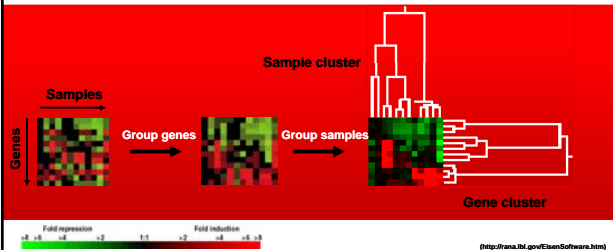
(Quackenbush, NEJM, 2004)

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Clustering

- Hierarchical clustering
 - ✓ Group samples and genes based on similarity



(<http://rana.lbl.gov/EisenSoftware.htm>)

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Application of expression microarrays

- **Experimental studies**
 - ✓ Genes affected by a given treatment
 - ✓ Chemical or physical, gene transfection, siRNA, etc.
 - ✓ Time series
- **Clinical studies**
 - ✓ Patterns of gene activity
 - ✓ .. can suggest a certain treatment regimen
 - ✓ .. response to a given treatment
 - ✓ Identification of novel candidate genes

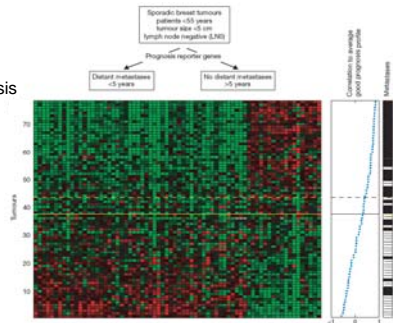
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Breast cancer

- Supervised classification

- ✓ Poor prognosis signature
- ✓ 70 genes



(van't Veer et al., Nature, 2002)

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Expression profiling with microarrays

Profiles for many cancer types

Signatures for

- ✓ New subgroups
- ✓ Metastasis
- ✓ Disease development
- ✓ Therapeutic response
- ✓

A large number of other diseases have been profiled

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Microarrays in clinical practice

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Agendia

- **MammaPrint**
 - ✓ 70-gene signature
 - ✓ Laboratory service
 - ✓ 2000 Euro
 - ✓ > 12,000 samples tested
 - ✓ FDA approval (Feb 2007)

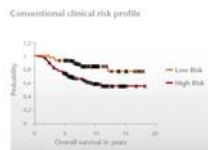
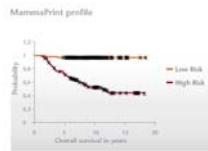
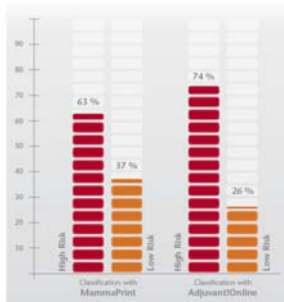


<http://www.agendia.com>

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MammaPrint



(Boysen et al., JNCI, 2006; <http://www.agendia.com>)

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Genomic Microarrays

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Genomic microarrays

Printing genomic DNA

- ✓ Whole BAC DNA
- ✓ PCR representations
- ✓ Oligonucleotides

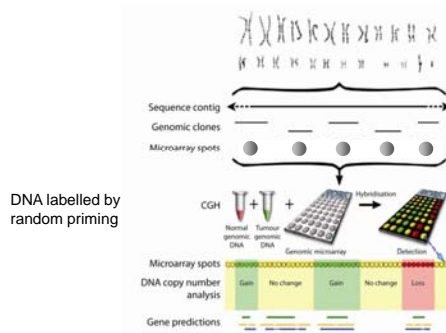
Measure copy number aberrations

- ✓ Gene amplification
- ✓ Deletions
- ✓ Translocation mapping
- ✓ Copy number variation (CNV)

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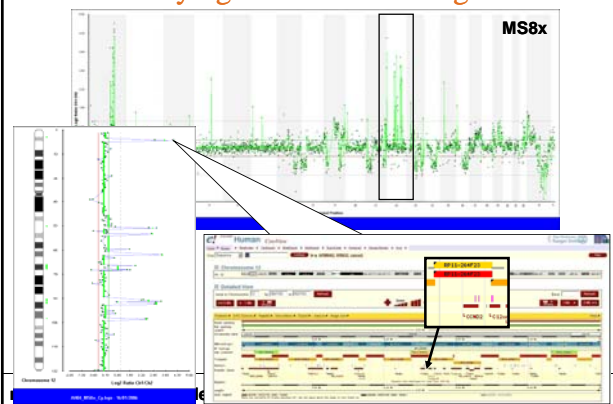
Array Comparative Genomic Hybridisation



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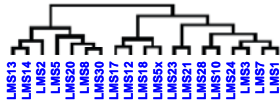


Identifying candidate cancer genes

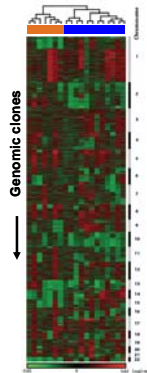


Sarcomas

- Sarcomas
 - ✓ Leiomyosarcomas
 - ✓ Gastrointestinal stromal tumours



DNA copy number changes reflexes the biology



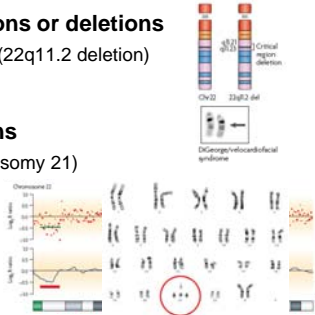
Meza-Zepeda et al., Cancer Res, 2006

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Constitutional genetic disorders

- Segmental duplications or deletions
 - ✓ DiGeorge syndrome (22q11.2 deletion)
 - ✓ ...
- Chromosomal regions
 - ✓ Down's syndrome (trisomy 21)
 - ✓ ...



(Emanuel & Saitta, Nat Rev Genet, 2007; <http://www.pediatrics.about.com>)

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Other types of Genomic Microarrays

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Systems Biology

Epigenetics

- DNA methylation
- Chromatin structure

Genome Structure

- DNA copy number

Genome Variation

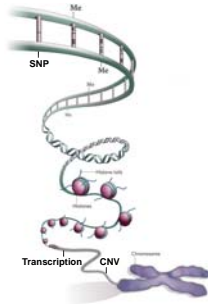
- SNP
- Copy number variation

Transcriptome

- mRNA expression
- miRNA expression
- Splice variance

Proteome

- Protein-Protein interactions
- DNA-protein interaction



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Sequencing an array of DNA molecules

Illumina/Solexa
Genome Analyzer



Applied Biosystems
SOLID Analyzer



Roche/454
Genome Analyzer FLX



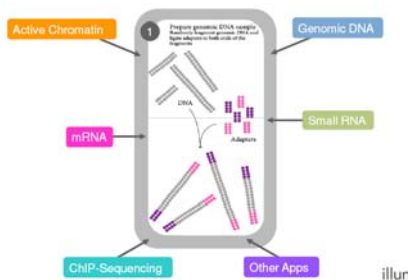
set

..... Also a number of upcoming technologies.

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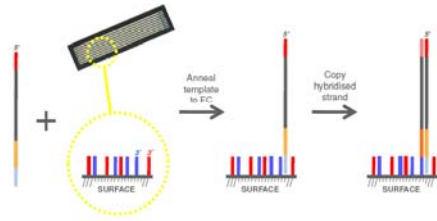
Sample preparation



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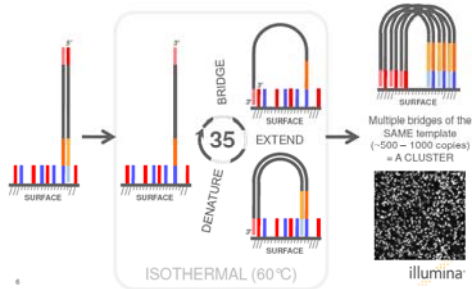
Cluster generation



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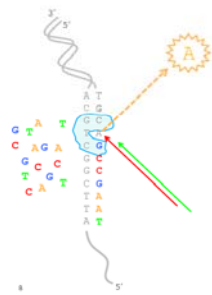
Cluster generation



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Sequencing by synthesis



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

illumina

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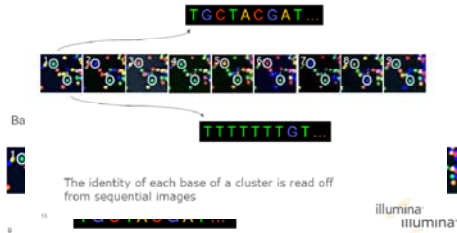


Reading the sequence

What SBS looks like

160 MILLION CLUSTERS
PER FLOW CELL

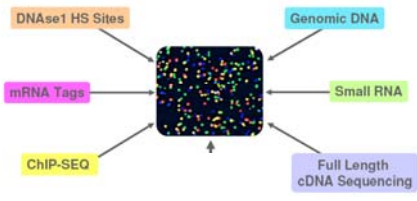
Base calling from raw data



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Applications



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Norwegian Microarray Consortium

Aim:

To make state-of-the-art microarray technology available to the scientific community at affordable cost

Means:

- Collaboration
- Efficient use of resources
- Avoid duplicate work
- Speed up establishment of advanced methodology



Made possible by a generous 3-year start-up grant from the Cancer Society and funds from host institutions



Currently running the FUGE microarray platform

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Service levels

- Experimental design (**Free**)
- Full data analysis: from raw data to gene lists, pathway analysis
- Partial data analysis
- More extensive analyses in collaboration with Bioinformatics Core

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Microarray Core Facility Complete Microarray Solutions



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oslo@microarray.no





Kreftforskningens nye høyborg!