

Genomics Core Facility



# Microarray technology and applications


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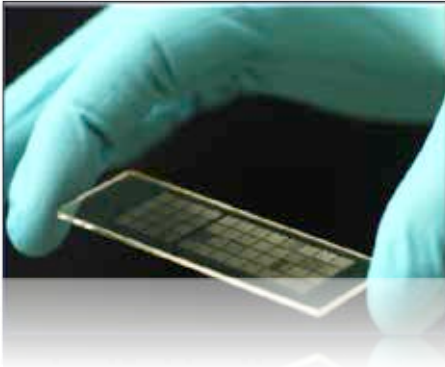
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# Microarray



Thousands of probe molecules at high-density onto a solid surface and use them to measure the amount of complementary molecules in complex mixtures

- DNA molecules, oligonucleotides
- Proteins
- Peptides
- Antibodies

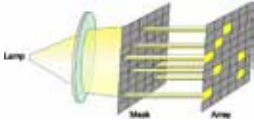
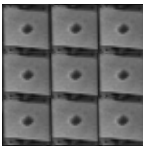
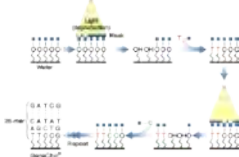
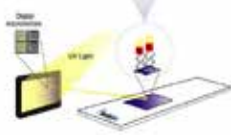
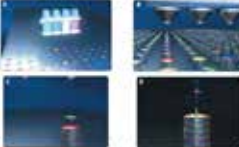
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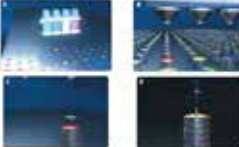

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## Oligonucleotide *in situ* synthesis


### Photolithography


### Inkjet

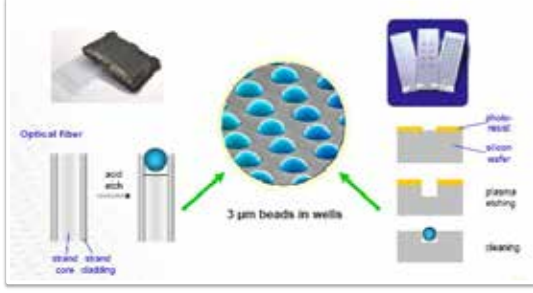



The Norwegian Radium Hospital



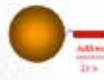
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## Oligonucleotide coted beads

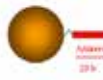





- Gene expression
- DNA methylation
- Genotyping
- DNA copy number




• GeneChip<sub>®</sub>\_CodonGain Assay &   
• QAGL Assay (QEX to TTPIC)  
• 25 base address code




• Expression\_Direct HiB-E  
• Whole Genome Genotyping\_31stream Assay  
• 50 base gene specific probe linked to Address  
• 25 base address code

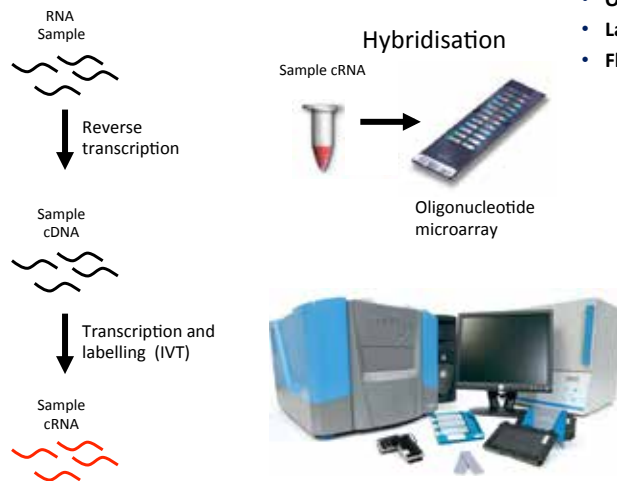




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## Single channel hybridisations



- One sample per array
- Labelled with Cy3 or biotin
- Flexible research design



## 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, etc.)

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

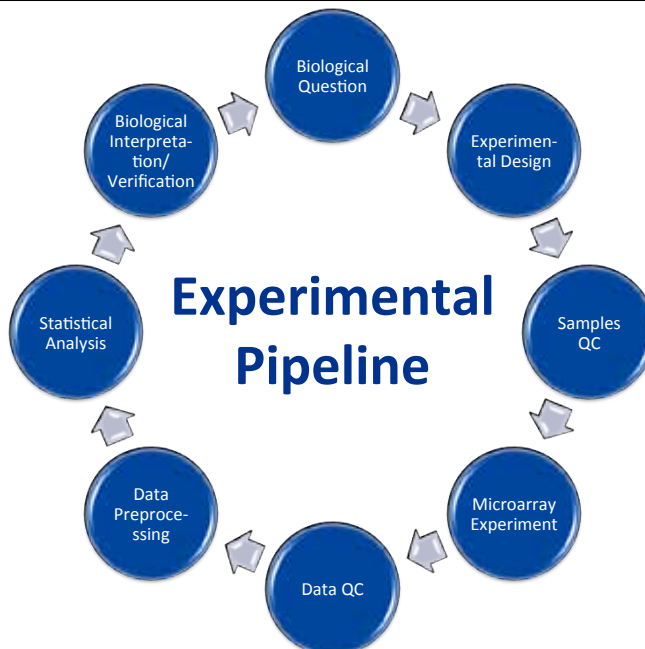
Today mainly using commercial oligonucleotide arrays

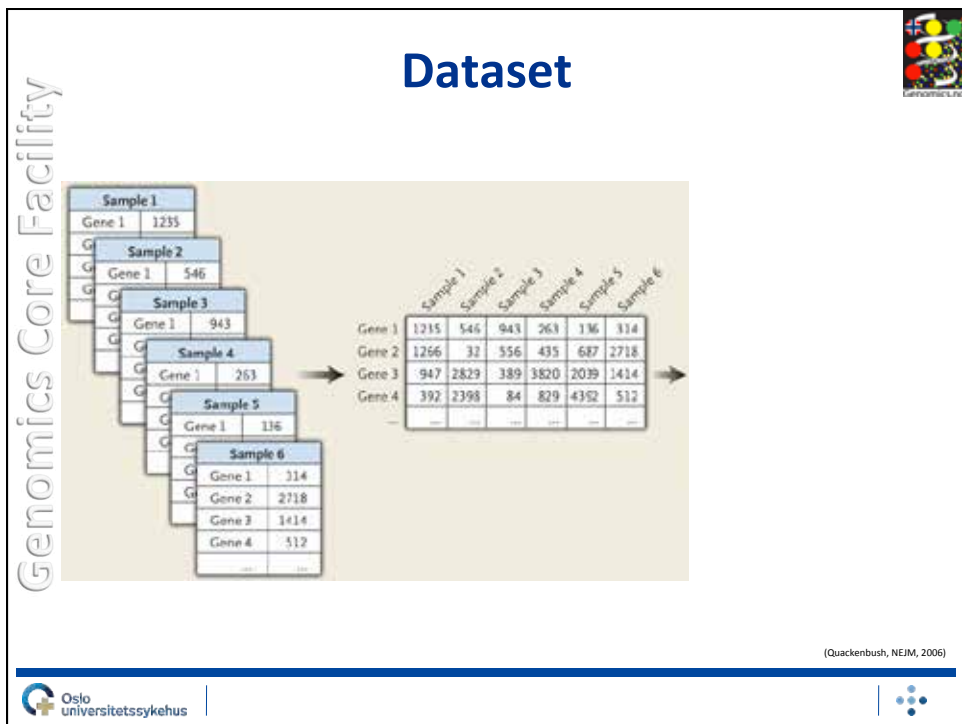
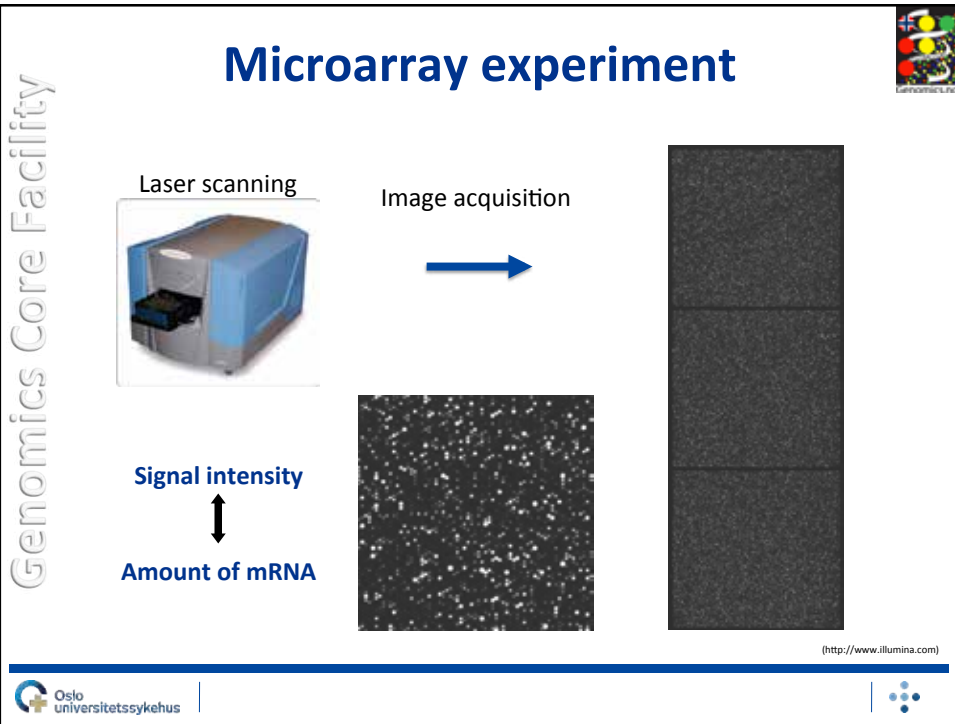


## Which platform to use?



- Organism
- Budget
- Amount of total RNA
- Number of samples
- Flexibility of the platform
  - Custom
  - Different levels of information



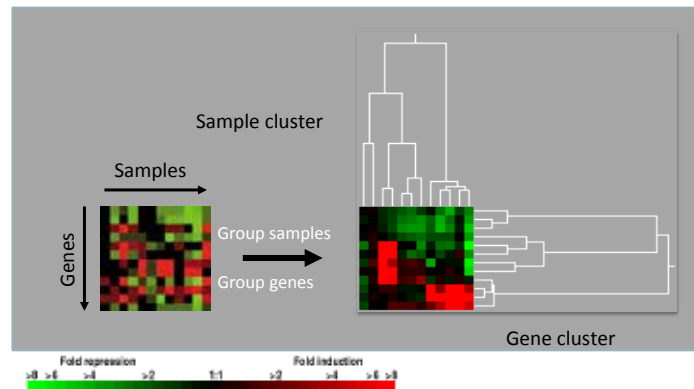


## Unsupervised Analysis



### Hierarchical clustering

Group samples and genes based on similarity



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



## Application of expression microarrays



### Experimental studies (Biological effects)

- Genes affected by a treatment
  - Chemical or physical, gene transfection, siRNA, etc.
- Time series response

### Clinical studies

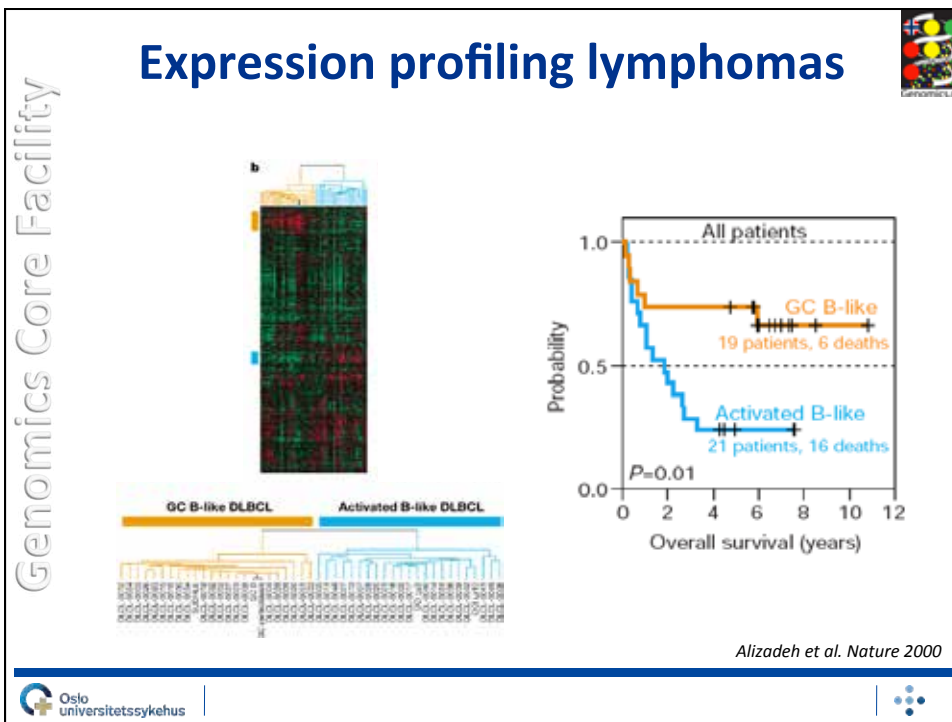
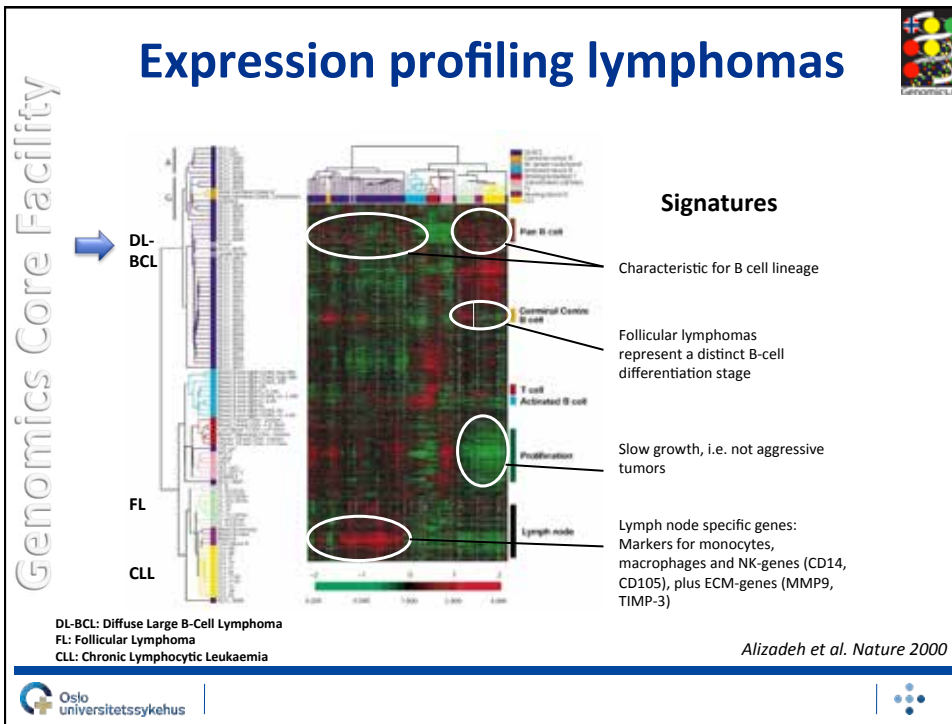
Patterns of gene activity

- Suggest a certain treatment regimen
- Response to a given treatment
- Drug resistance

Identification of novel candidate genes

- Biomarkers
- Target for therapy

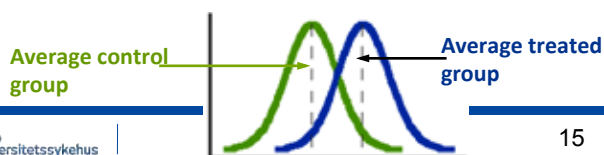




## Supervised analysis Differentially expressed genes



- **Aim:** Find differentially expressed genes between two groups
  - **Groups:** Different treatment, time points, phenotypes, etc.
- **What is differential expression?**
  - Measurements before (1.5, 0.8, 1.2) and after (2.1, 1.7, 1.5) treatment
  - Are the groups statistically different?
    - Requires a model to help estimate this difference
      - i.e. T-statistics, modify for multiple testing



## Breast cancer

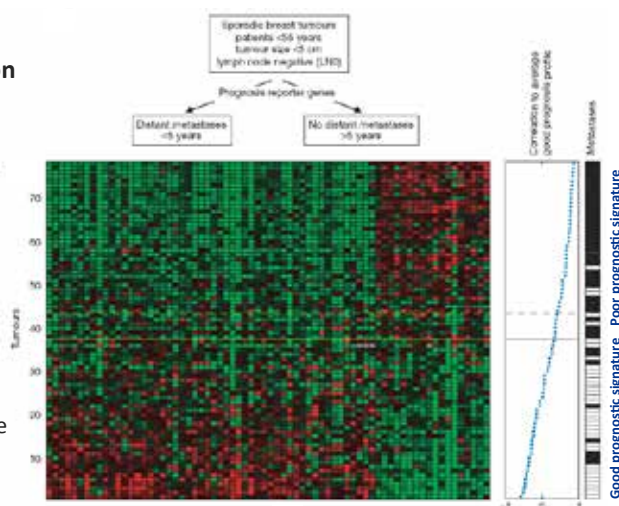


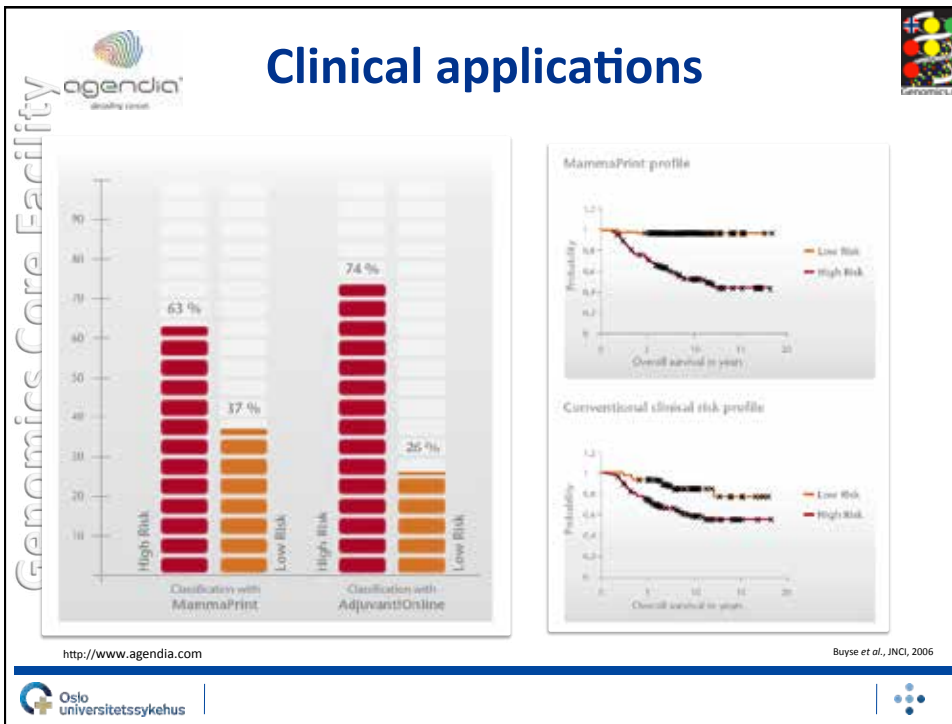
### Supervised classification

- Two groups
- With metastasis
  - Without metastasis

### Which genes can differentiate these groups?

- 70 genes
- Poor prognosis signature





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DIAGENIC

## Early detection

**Alzheimer Disease early detection**  
**ADtect®** is a blood test to aid in the early detection of Alzheimer's disease. Detects changes in the gene activity of 96 genes. These changes are disease specific.

*Rye PD et al, J Alzheimers Dis. 2011*

**Breast Cancer early detection**  
**BCtect®** is a blood test to aid in the early detection of breast cancer. Detects changes in the gene activity of 96 genes. These changes are disease specific and BCtect® is able to detect early stage breast cancer.

*Aarøe J et al, Breast Cancer Res. 2010*

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## Gene signatures

### Information

- Molecular mechanisms involved in physiologic or pathologic processes

### Identification

- New genes involved in a pathway or disease
- Potential therapeutic targets
- Biomarker

### Classification

- New biological subgroups
- Stratification of samples or patients



## Genomic Microarrays



## Genomic microarrays



### Genomic representation in an array format

#### Printing genomic DNA

- PAC or BAC DNA
- cDNAs
- Oligonucleotides (long and SNP)

#### Measure copy number aberrations

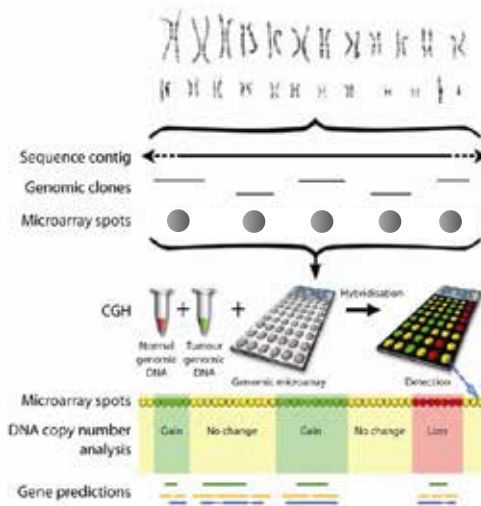
- Gene amplification
- Deletions
- Copy number variation (CNV)

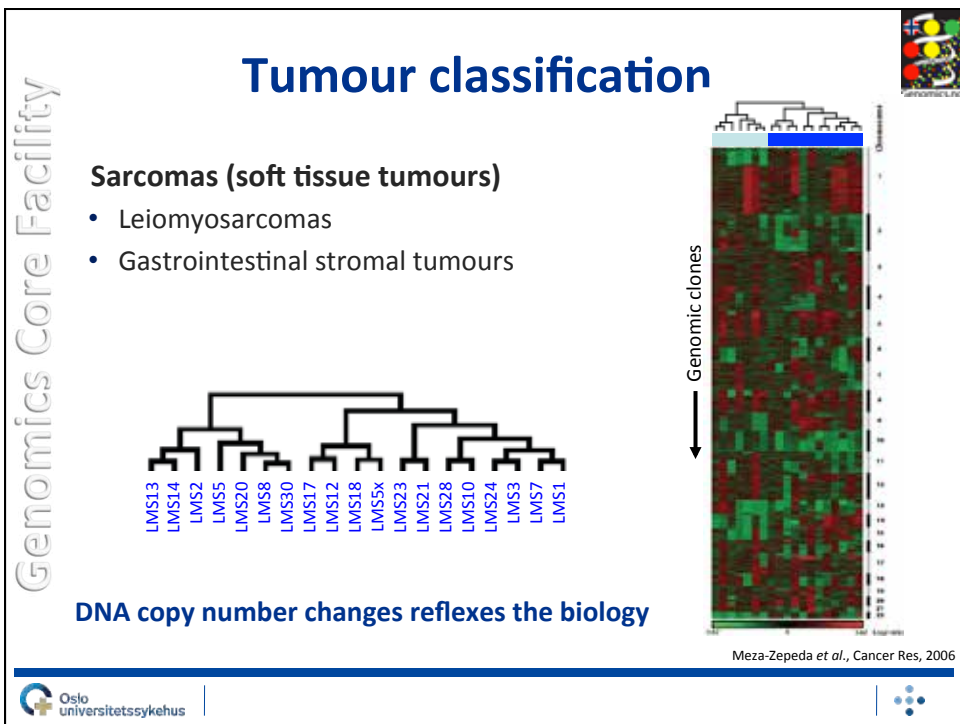
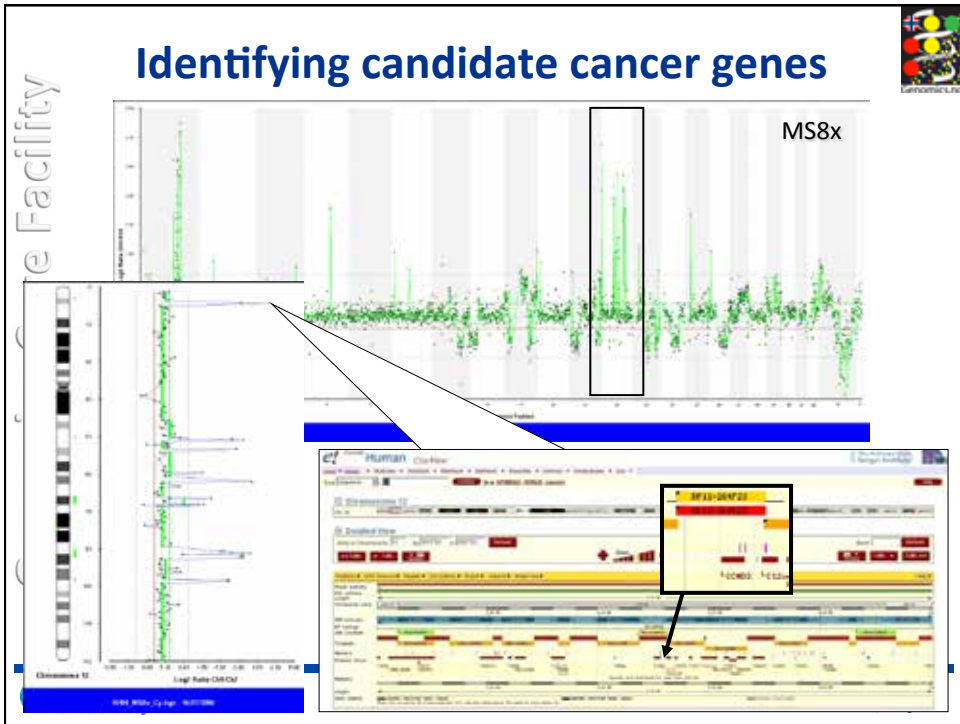


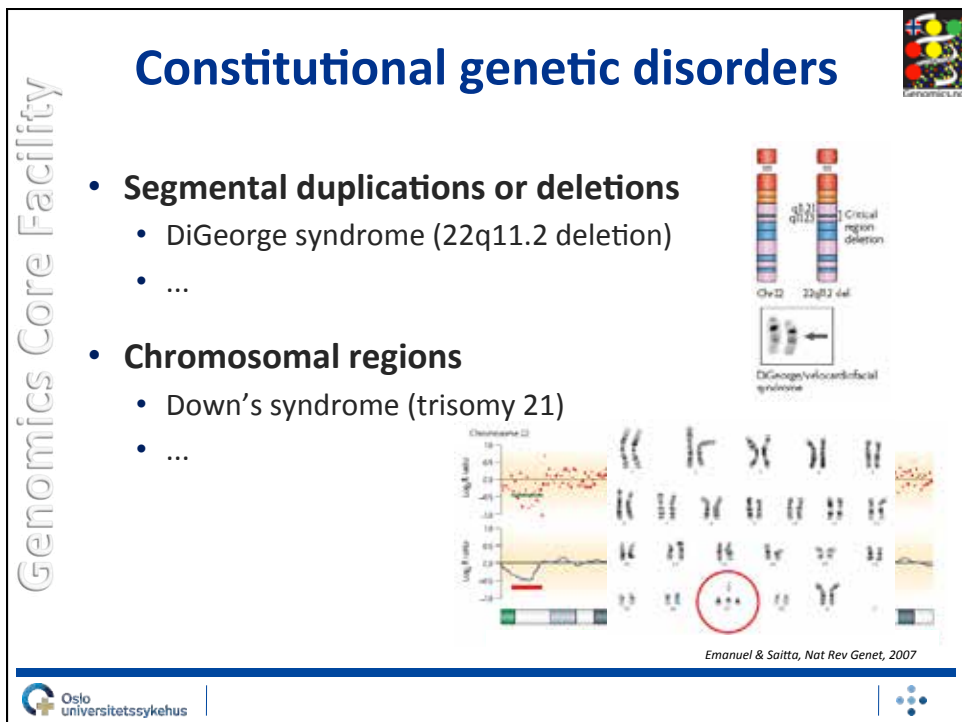
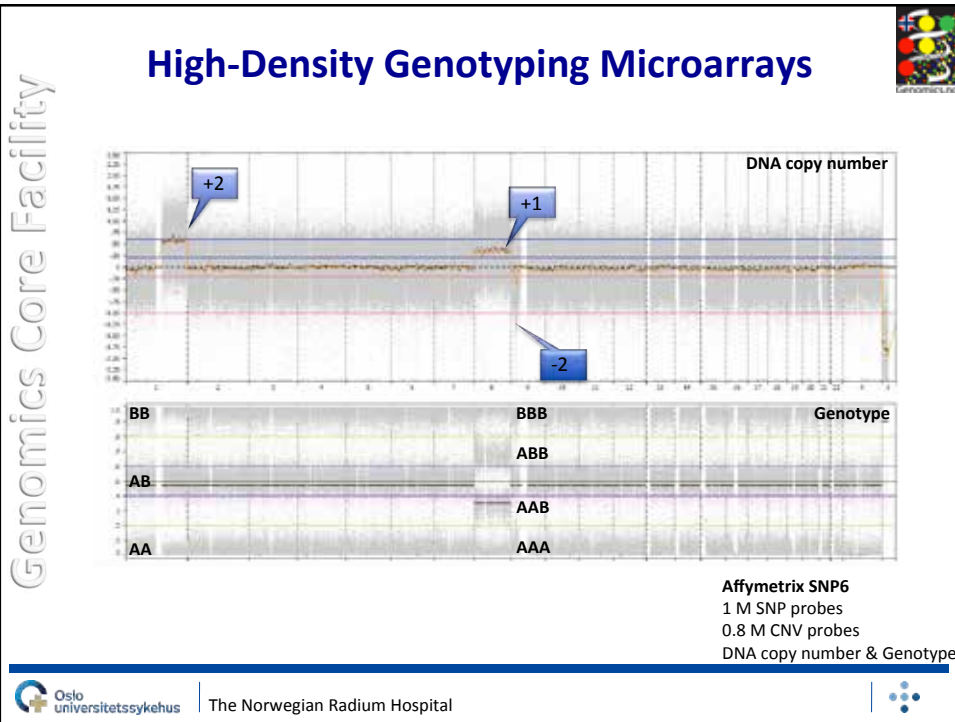
## Array Comparative Genomic Hybridisation



DNA labelled by random priming





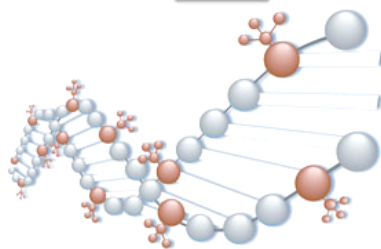
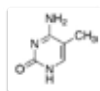




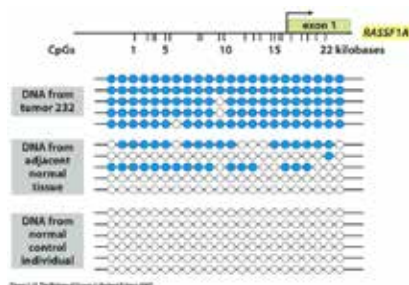
# Other applications



# CpG Island Methylation



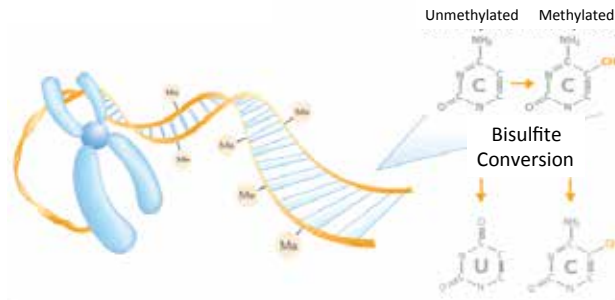
Gene silencing



Genome-wide methylation maps



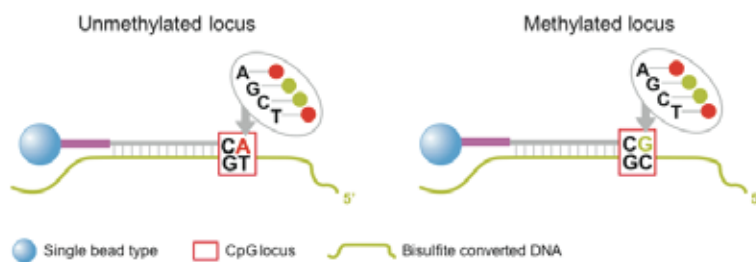
## Bisulfite Conversion

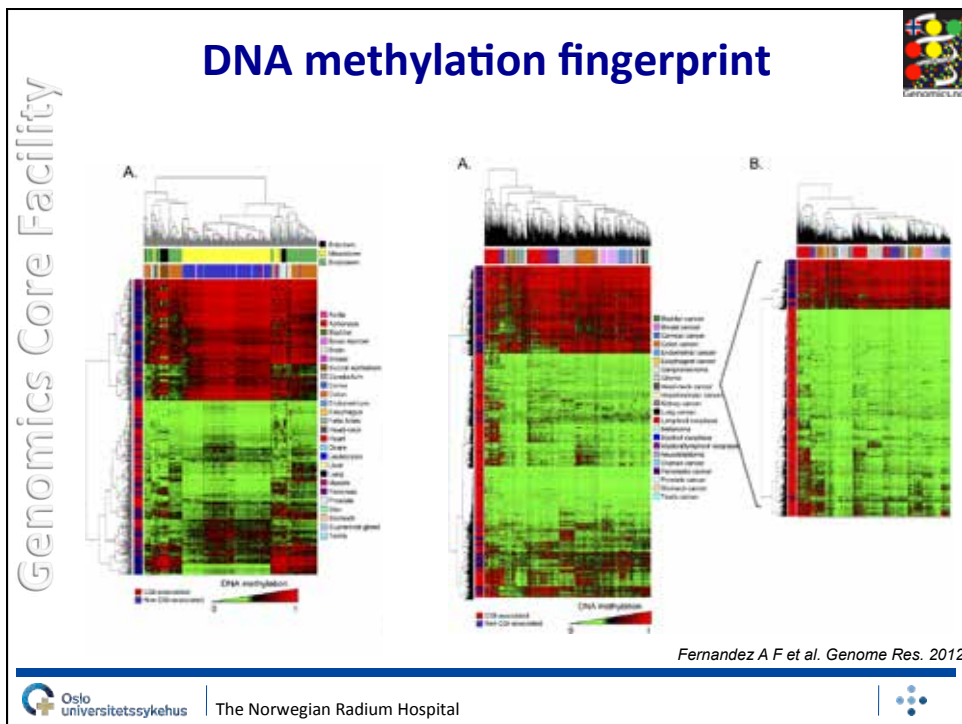
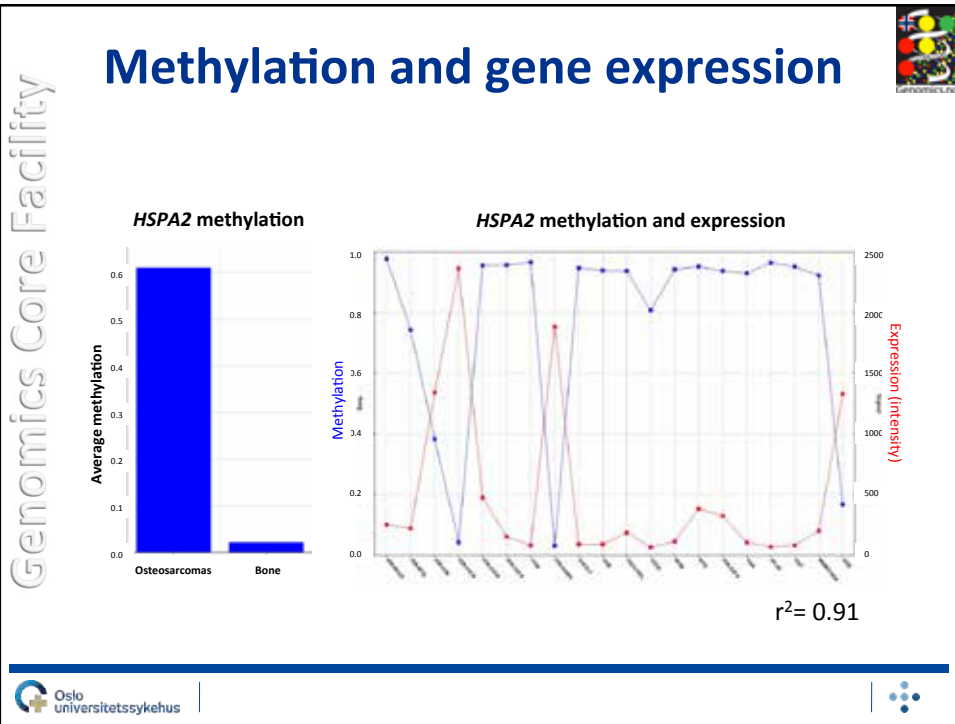


**Methylated cytosines remains a cytosines**  
**Unmethylated cytosines convert to uracils**



## Illumina Infinium Technology

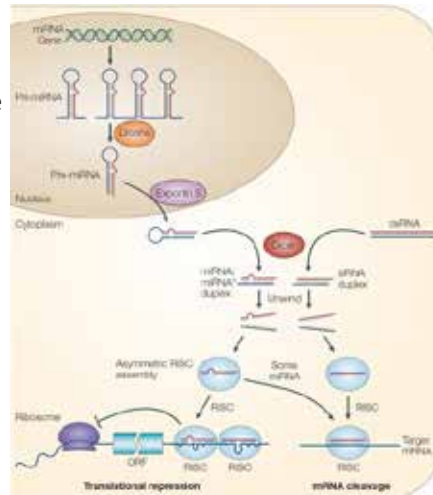




## miRNAs

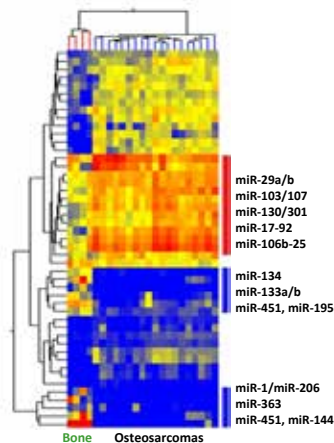
### miRNAs

- Non-coding RNAs which regulate gene expression
- 20-22 bp
- Involved in various biological processes, e.g. development, differentiation, apoptosis and proliferation
- Acts by translational inhibition or mRNA degradation

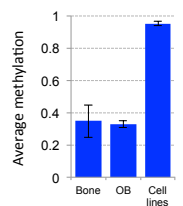


## miRNAs expression

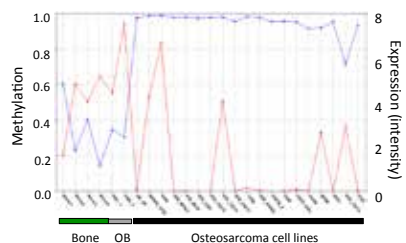
### miRNA Gene Expression



### miR-335 methylation



### miR-335 methylation and expression



Heidi Namlos et al

# Systems Biology



## Epigenetics

- DNA methylation
- Chromatin structure

## Genome Structure

- DNA copy number changes

## Genome Variation

- SNP
- Copy number variation

## Transcriptome

- mRNA expression
- miRNA expression
- Splice variance

## Proteome

- Protein-Protein interactions
- DNA-protein interaction

