

Different microarray applications

Aim of lecture: To get some basic knowledge about the different applications microarrays are used for

Rita Holdhus
Introduction to microarrays
December 2011

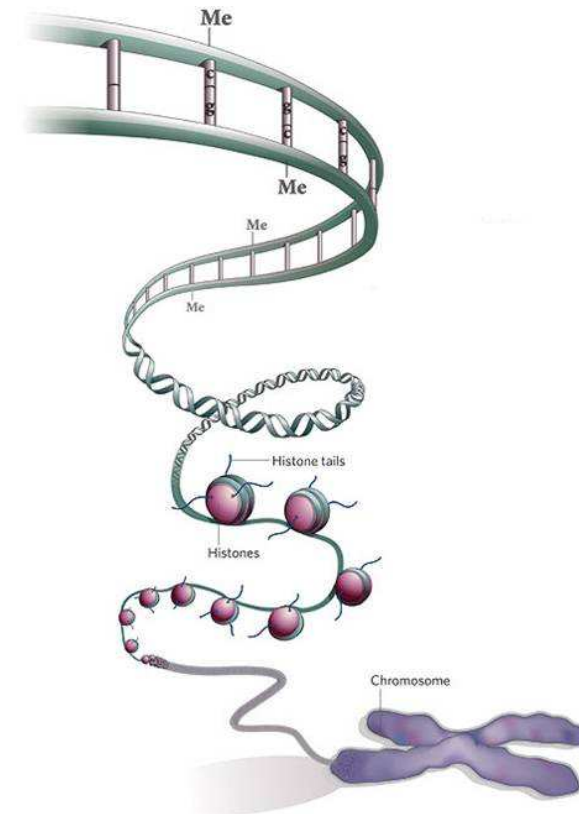
microarray.no



Systems biology – microarray applications

The understanding of a biological system requires knowledge of the molecular mechanisms involved in regulation at:

DNA level	<ul style="list-style-type: none">• DNA Copy Number• SNP
RNA level	<ul style="list-style-type: none">• Gene expression• miRNA expression
Protein level	<ul style="list-style-type: none">• ChIP on chip• Protein arrays



microarray.no



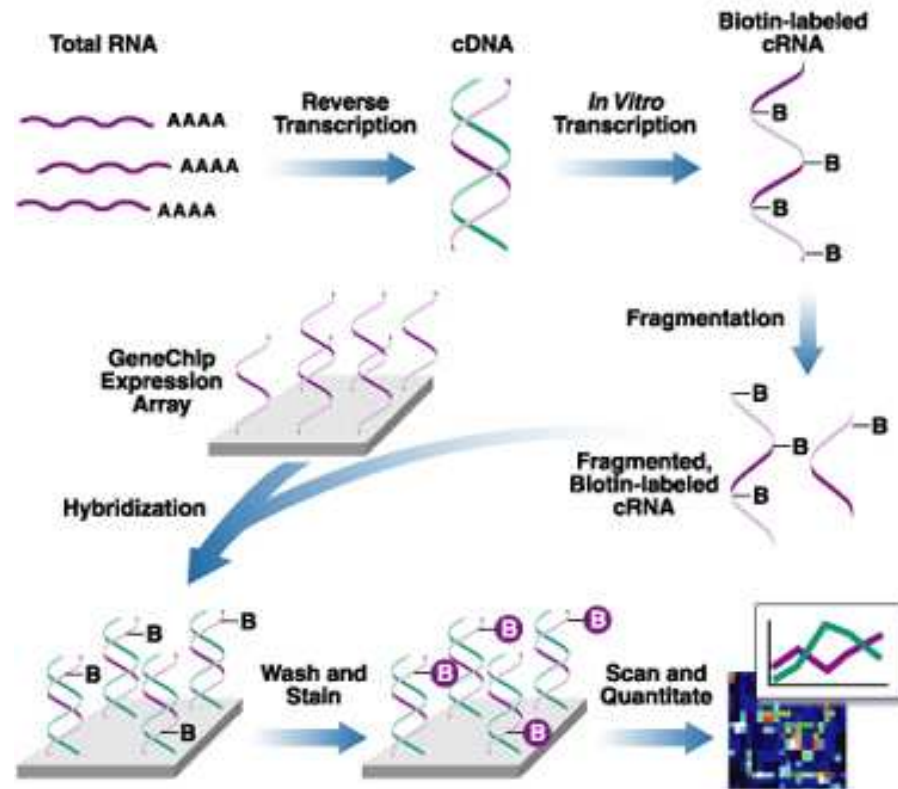
Gene expression

Measuring mRNA transcripts in order to determine the gene expression profile in the sample

Monitoring thousands of genes simultaneously

Only known genes or ESTs can be analyzed

Measuring: RNA in order to find gene expression changes

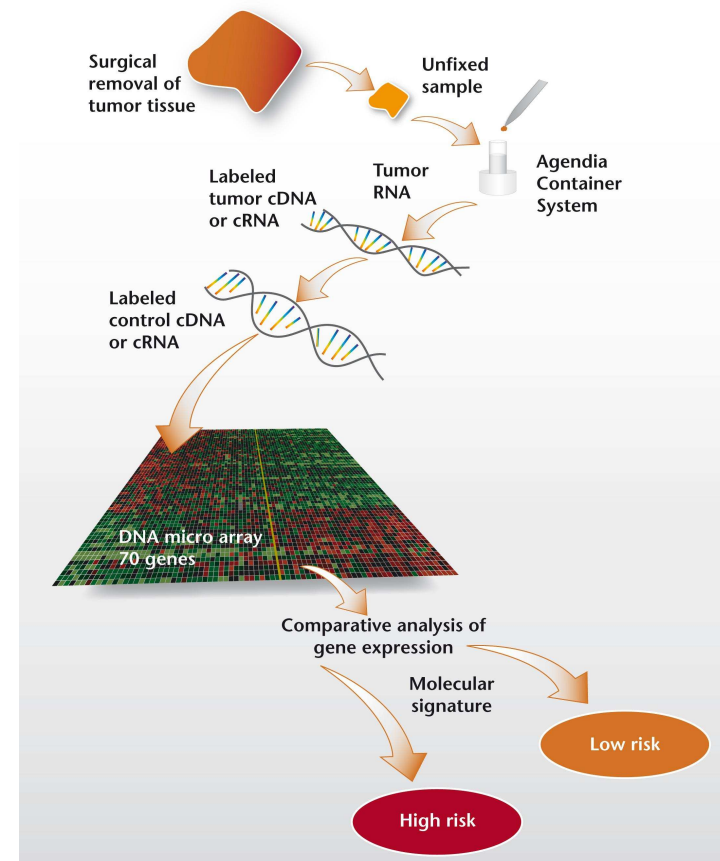


microarray.no



Gene expression studies

- Gene affected by a given treatment
- Time series (with and without a given treatment)
- Patterns of gene activity (healthy vs. control)
- Predicting disease by gene patterns
 - Predict outcome of AML disease
 - MammaPrint (breast cancer prognosis)



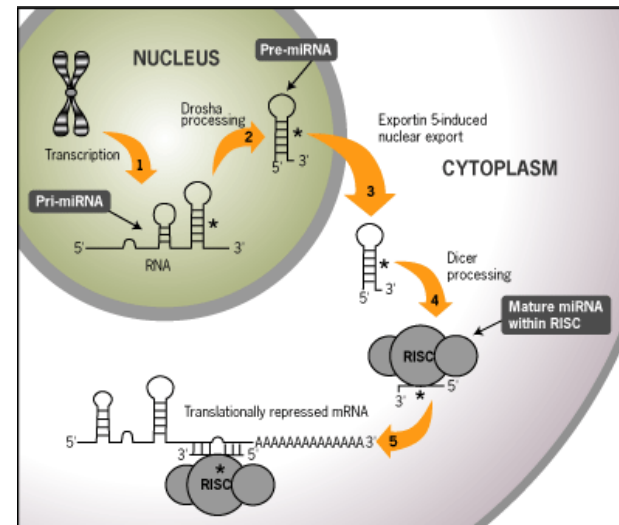
microarray.no

<http://www.agendia.com/pages/mammaprint/21.php>



miRNA

- miRNA are small non-coding RNAs (19-23 nucleotides)
- miRNA act as regulators that binds to complementary sequences in the 3'UTR of a target mRNA transcript
- Over 10 000 miRNAs have been identified in a range of species, 940 found in humans
- Estimated that more than 60% of human protein-encoding genes harbor miRNA target sites



microarray.no

Measuring: RNA in order to determine miRNA expression profiles

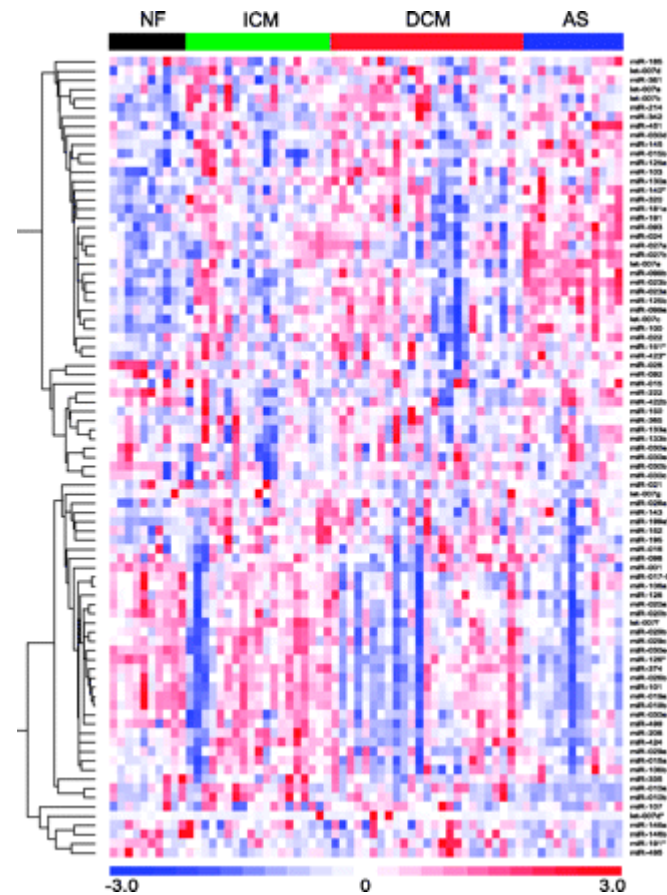
miRNA study

Human heart failure

A study of miRNA expression in 67 human left ventricular heart samples

Out of 87 detected miRNAs, 43 were differentially expressed in at least one disease group, suggesting that miRNA expression is altered in heart disease

miRNA expression pattern is distinct between diagnostic classes



microarray.no



miRNA

miRNA have shown to be

- Critical in the development of organisms
- Negative regulators of mRNA translation
- Differentially expressed in tissues
- Involved in viral infection processes
- Associated with oncogenes

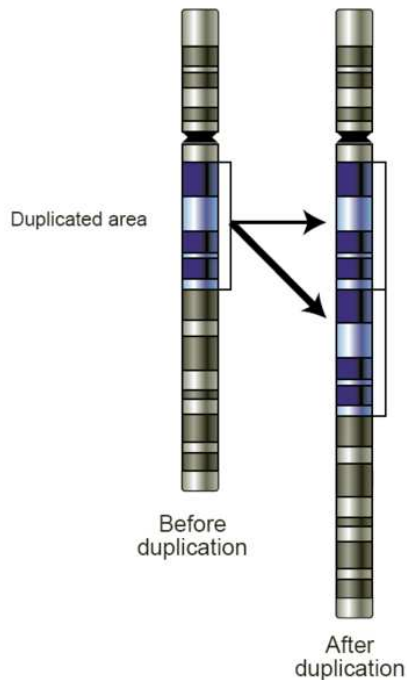
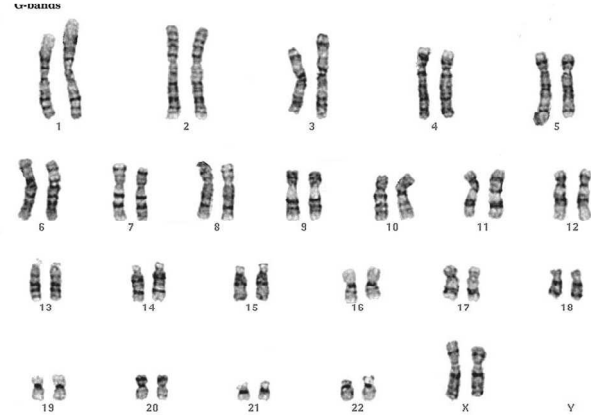
Potential in miRNA expression profiling

- Insight in gene regulation mechanisms
- Biological subgrouping (diagnosis and prognosis)
- Identification of potential therapeutic miRNA targets



DNA copy number

A normal somatic cell has two copies of each chromosome



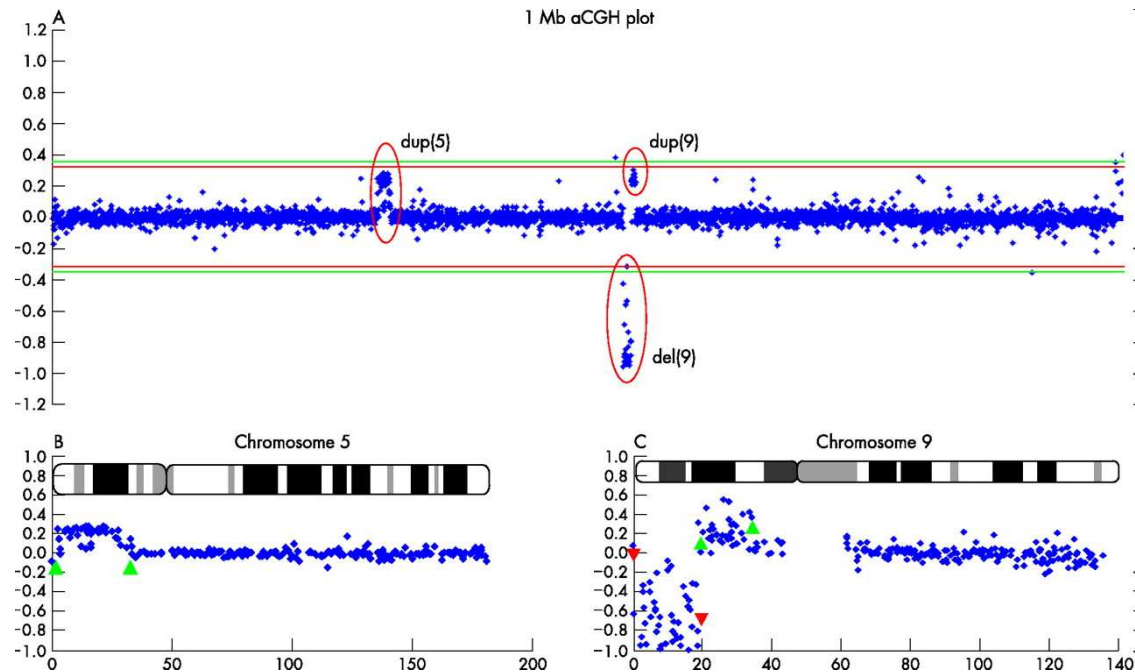
An alteration of the DNA of a genome that results in the cell having an abnormal number of copies of one or more sections of the DNA

Caused by structural rearrangements in the genome such as deletions, duplications, inversions and translocations

microarray.no



DNA copy number changes can be detected using cytogenetic techniques such as FISH, CGH, karyotyping and SNP arrays



microarray.no

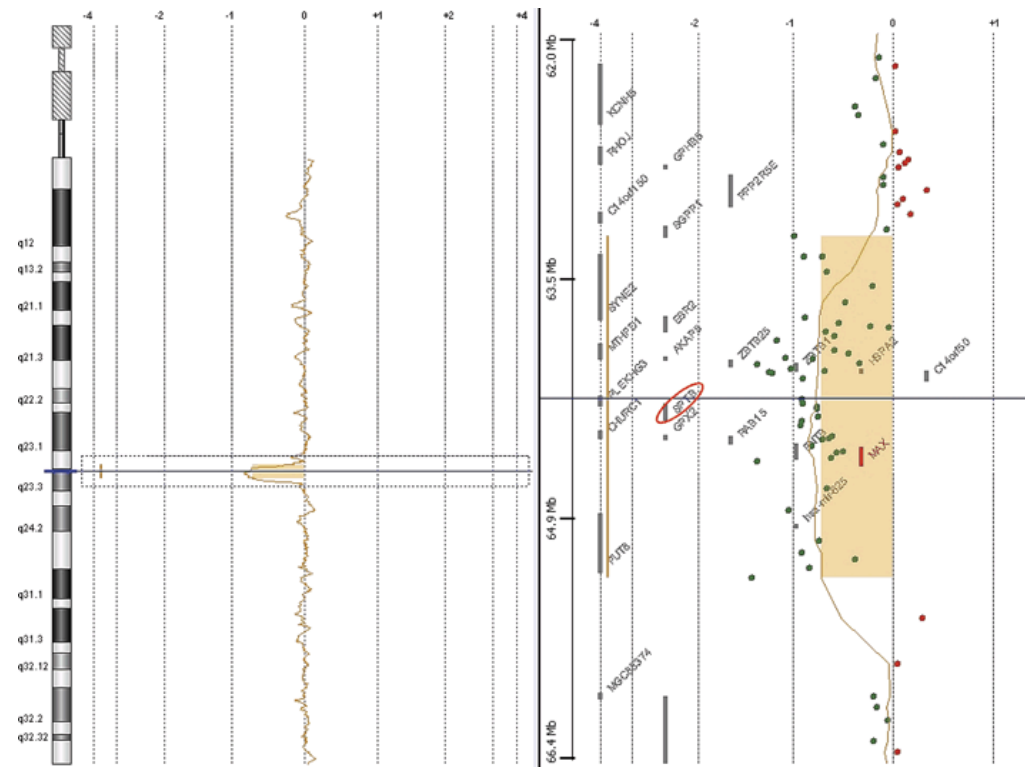
Measuring: DNA in order to find DNA copy number changes



DNA copy number study

Potential in DNA copy number profiling

- Identification of candidate (disease) genes
- Identification of genetic disorders/syndromes



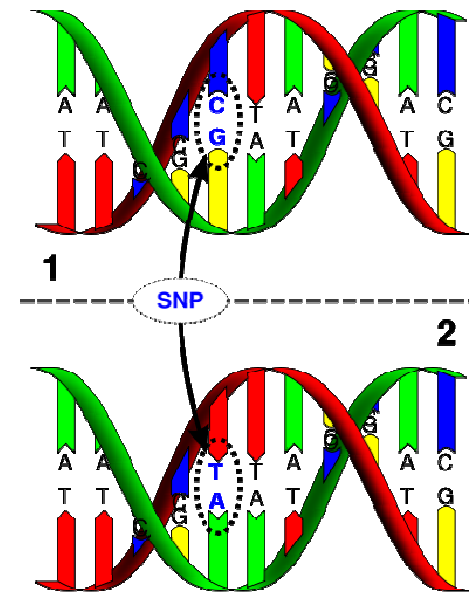
Lybæk et al., clin Genet 2008

microarray.no



SNP

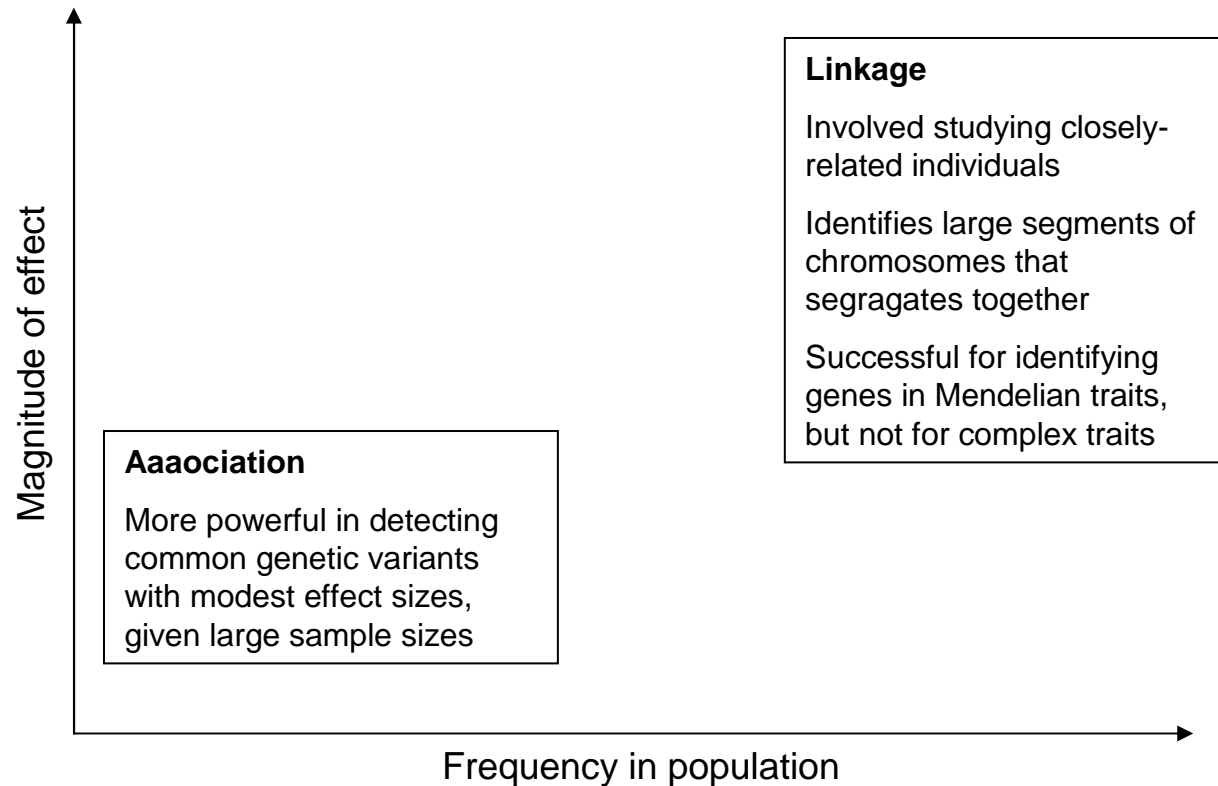
- SNPs are normally occurring DNA variants (markers) located in the genome
- SNPs are DNA sequence variations occurring when a single nucleotide –A, T, C or G in the genome differs between members of a species (or between paired chromosomes in an individual)
- SNP-genotyping is used to study diseases since SNP are inheritable.
- Genotyping is used for identifying novel candidate (disease) genes and mapping multifactorial conditions/diseases



microarray.no

Measuring: DNA in order to determine the SNP-genotype profile

Two types of SNP studies

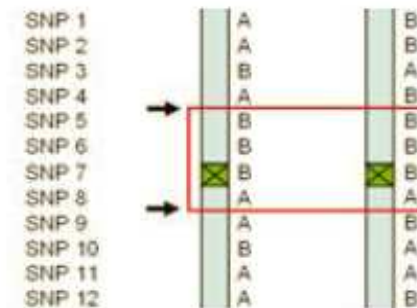
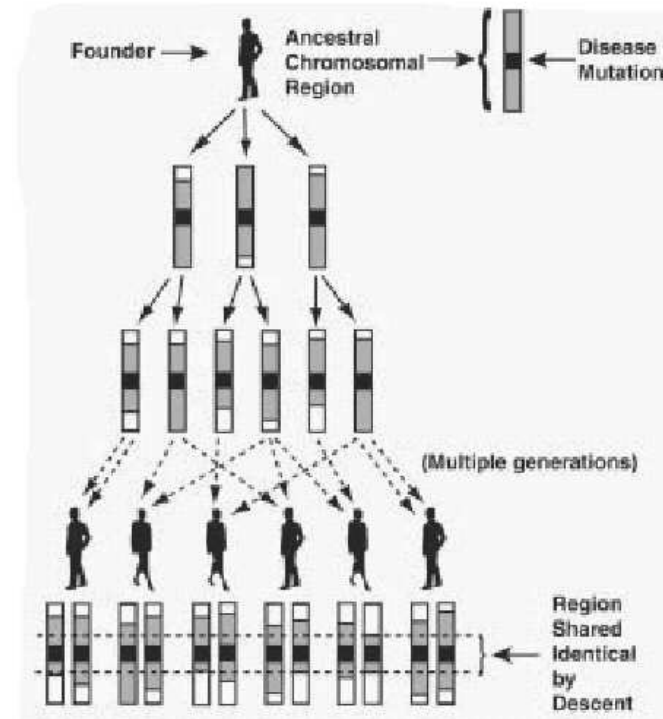


microarray.no



SNP – linkage studies

- Linkage study is a method that allows us to determine regions of chromosomes that are likely to contain a risk gene, and rule out areas where there is a low chance of finding a risk gene
- The linkage method works by linking a given SNP-genotype to a given disease and thereby to a DNA region containing the disease causing gene.
- Patients needs to be related to each other.



microarray.no



SNP - Linkage study

microarray.no

20 NYHET

LØRDAG 25. SEPTEMBER 2004 Dagbladet



GENFEIL: Etter snart 22 års kamp har Nan-Therese Hermansen endelig fått svar. En genfeil utløser de voldsomme svetteanfallet. Foto: Tore Bergsaker

Svetteanfallet skyldes genfeil

Etter 22 år med vandringsvettige svetteanfallet, har Nan-Therese Hermansen fått nytt håp om at lidelsene snart skal ta slutt. Norske genforskere har funnet feilen.

Tekst: Hilde Schjerve
h.sj@dagbladet.no

– Det er bekjærtelig! Jeg dro rett ut og fetes med femteets mid- dag sammen med pappa, sier Nan-Therese Hermansen til Dagbladet.

Genfeil
Ingen diagnose, ingen behandling. Trass i usalige urechninger var Nan-Therese forsket en gåse for

legene da hun sto fram i Dagbladet i mars.

Nå har derimot en professor i Bergen løst gåsen. Nan-Therese svetteanfallet skyldes en feil på ett av hennes 20 000 gener.

– Klart det er morsomt å ha funnet hemmeligheten! Hadde det ikke vært for at Nan-Therese sto fram i Dagbladet, ville hun kanskje vært til utredning for epilepsi og hatt en mye lengre vei å gå fram mot riktig diagnose, sier professor og overlege Helge Boman, ved Senter for medisinsk genetik og molekylærmedisin ved Helsekand universitetssykehus.



GLEDELIG: Nan-Therese Hermansen og seksjonsoverlege Erik Sætre gleder seg over nyheten. Foto: Tore Bergsaker

De to har aldri møttes. Både blodprøver fra Nan-Therese og hennes foreldre ble sendt over fjellet. Genetaken kunne starte. Forskerne jobbet ut fra hypotesen om at Nan-Therese lider av kaldeindusert svette- syndrom (KISS). I fjor fant Boman forskerteam genfeilen som er årsaken til KISS, i samarbeid med israelske og amerikanske kolleger.

– KISS er et nytt sjeldent Boman- gruppe har funnet genfeil bare hos en håndfull pasienter: To søstre fra Israel, tre fra Norge, en

fra Canada, en fra Australia og nå Nan-Therese. Årsaken til genfeilen er en mutasjon som Nan-Therese har arvet fra begge foreldrene. De hadde en risiko for at en av fire barn skulle få ubalansen.

– Det store spørsmålet nå er om det finnes noen behandling som kan hjelpe 22-åringen. Hun har blitt skuffet nok tidligere, så jeg vil ikke gi henne for mye håp, men en kanadisk professor i nevrologi har funnet fram til tiltak som har hjulpet hennes pasient. Av en eller annen grunn droyer det før jeg får vite hva be-

handlingen går ut på, sier Boman. Siden han ikke er Nan-Therese behandlende lege, vil han ikke blande seg bort hva Ullevål universitetssykehus bestemmer seg for.

– Jeg har foreskrevet meg om at norske KISS-pasienter vil være velkomne til utredning i Canada, dersom det er et ønskelig, sier Boman.

Nan-Therese reiser nå som helst. – Det er godt bare å få vite hva som er feil med meg. Men jeg ser ikke håp på for mye. Finnet de en overraskelse om et nederlag. Jeg må være realistisk, sier hun.

- Linkage study identified the gene causing Cold-Induced Sweating Syndrome (CISS)

- CISS is a very rare disease and has only been detected in 8 individuals from 5 different families

- Nan-Therese inherited the same mutated gene-allele from both her parents

Knappskog et al., AM J Hum Genet 2003



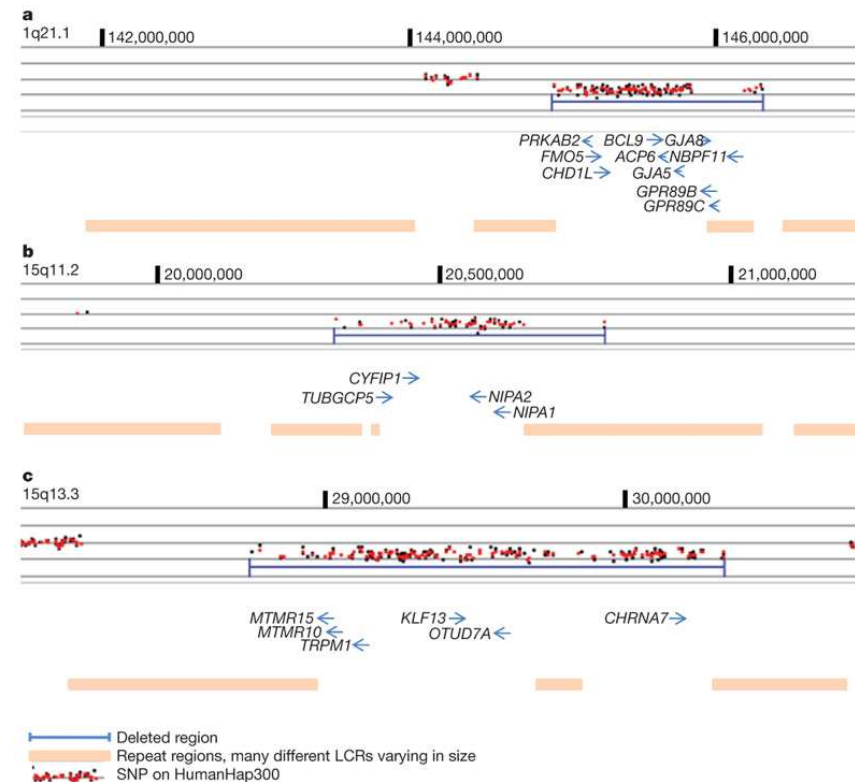
Whole genome association studies (GWAS)

- Association study: Examination of many common genetic variants on different individuals to see if any variant is associated with a trait
- Association studies are used in so-called "common public health diseases" as:
 - Diabetes
 - Rheumatism
 - Heart-/stroke diseases
 - Psychological diseases
- Such diseases are "multifactorial" i.e. influenced by both genetic- and environmental factors.
- Therefore association studies requires thousands of patients with a certain disease and thousands of control samples



Association studies

- SNP-genotyping of genes involved in development of schizophrenia
 - 4 718 persons with schizophrenia
 - 41 201 control persons
 - Individuals included from 10 countries
- Found deletions that were 12-15 times more frequent in persons with schizophrenia than in control persons
- Indicates that the genes located in these deletions are involved in development of schizophrenia

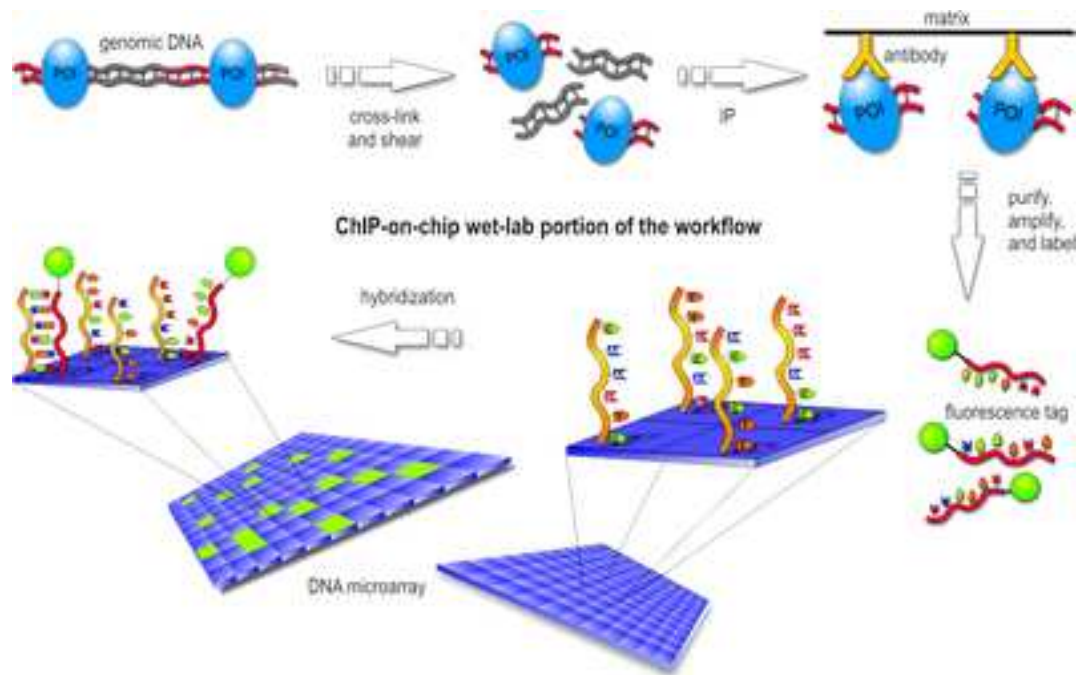


microarray.no



chIP-on-chip

- chIP-on-chip: chromatin immunoprecipitation-on-chip
- Immunoprecipitation of DNA-binding proteins and their target DNA sequences
- Regulatory proteins binds to promotor DNA regions and thereby regulate gene expression



microarray.no

Measuring: DNA fragments that has been bound to a specific protein

Goal of ChIP-on-chip is to localize proteins binding sites that may help identify functional elements in the genome

Promotor regions

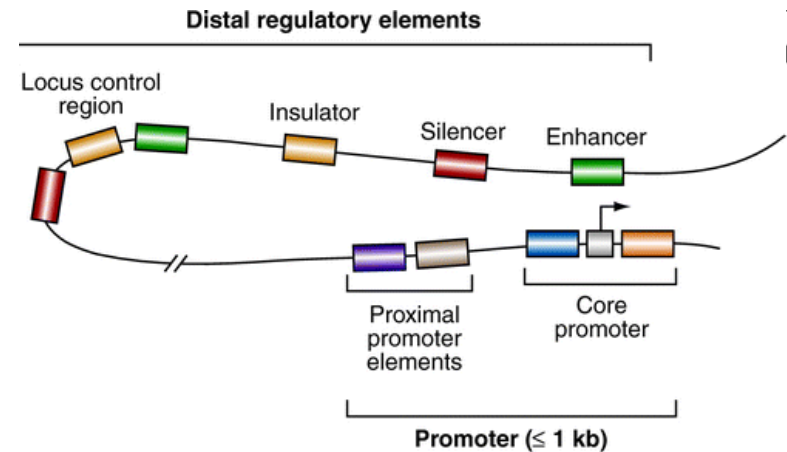
Enhancers

Repressors

Silencing elements

Insulators

Sequences that control DNA replication



Some drawbacks

Expensive for genome-wide resolution (need tiling arrays)

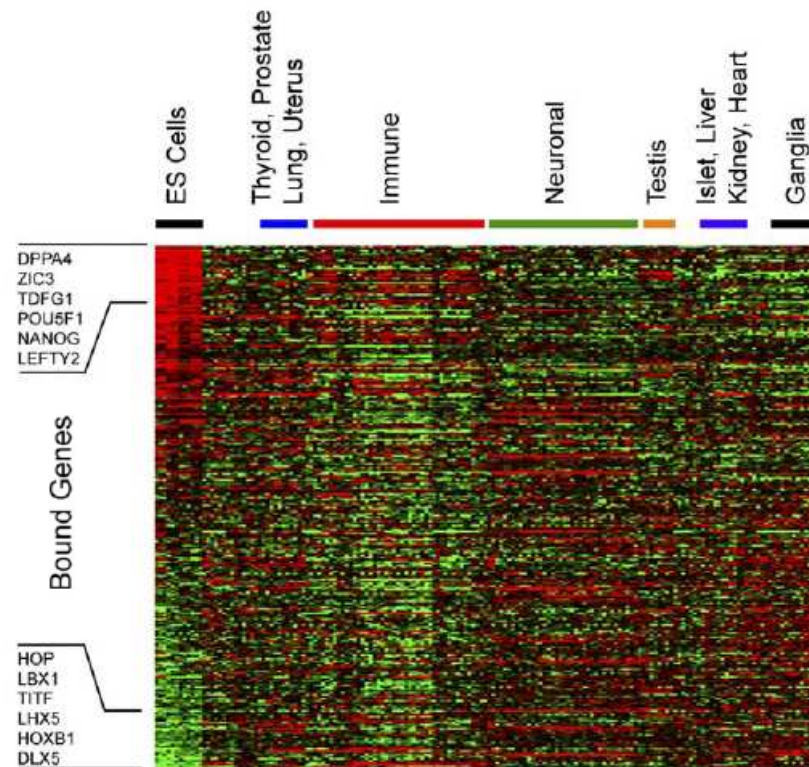
Need highly specific antibodies

Resolution (30-100bp)



chIP-on-chip

- Study of transcription factors and gene expression in human embryonic stem cells vs. in differentiated tissues and cell types
- Identification of target genes for three stem cell specific transcription factors



microarray.no



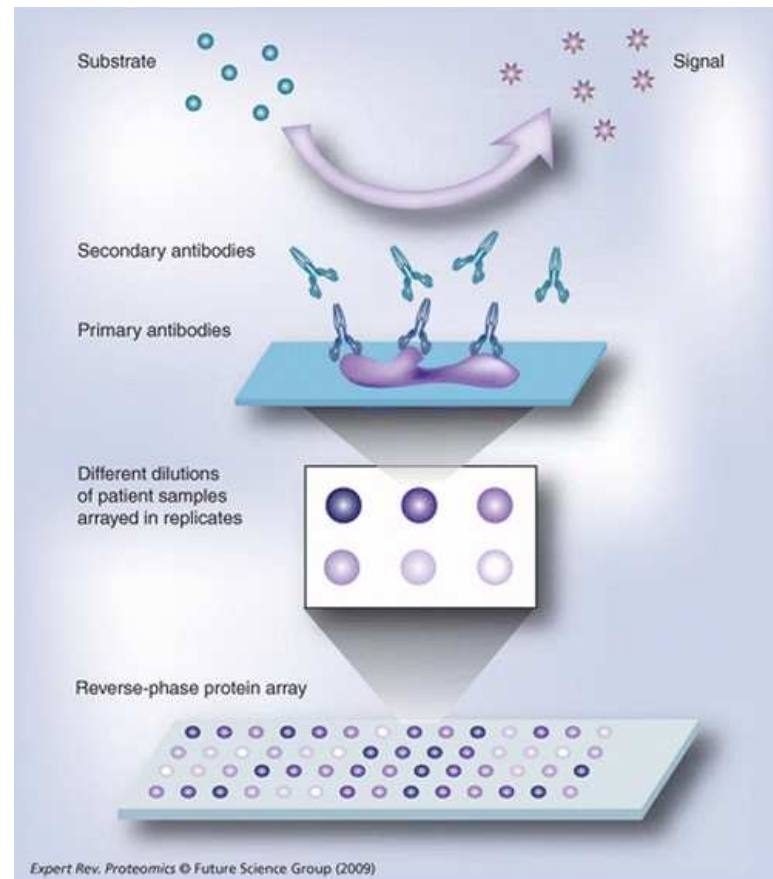
Protein arrays

- Screening of thousands of different interactions:
 - Protein - antibody
 - Protein – protein
 - Protein – ligand
 - Protein – drug
 - Enzyme – substrate

Drawbacks

Antibodies

Different proteins like
different solution conditions



microarray.no

Systems biology – microarray applications

Transcriptome

- mRNA expression
- miRNA expression
- Splice variance

Genome structure

- DNA copy number

Genome variation

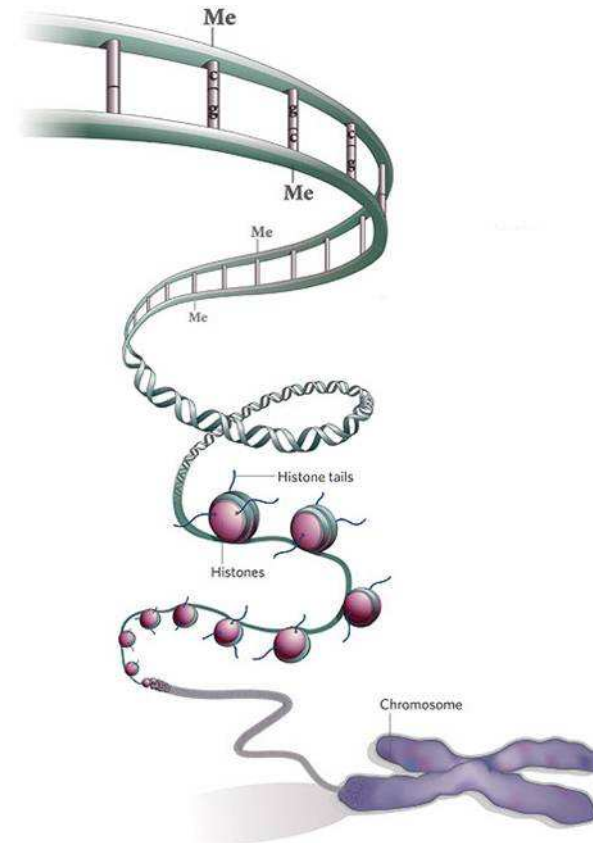
- Single nucleotide polymorphism
- Copy number variations

Proteome

- Protein-protein interactions
- DNA-protein interaction

Epigenetics

- DNA Methylation
- Chromatin structure



microarray.no