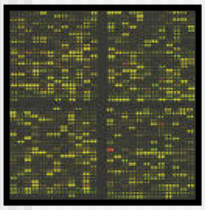
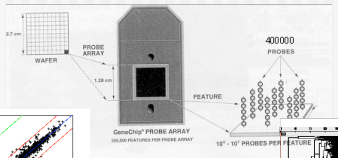


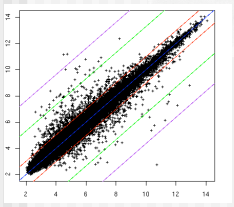
Microarray Informatics

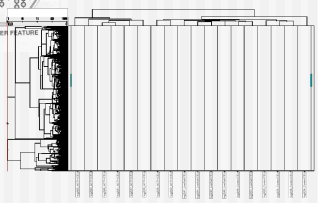
Donald Dunbar

MSc Seminar

4th February 2009







Aims

- To give a biologist's view of microarray experiments
- To explain the technologies involved
- To describe typical microarray experiments
- To show how to get the most from and experiment
- To show where the field is going

Introduction

- Part 1
  - Microarrays in biological research
  - A typical microarray experiment
  - Experiment design, data pre-processing
- Part 2
  - Data analysis and mining
  - Microarray standards and resources
  - Recent advances

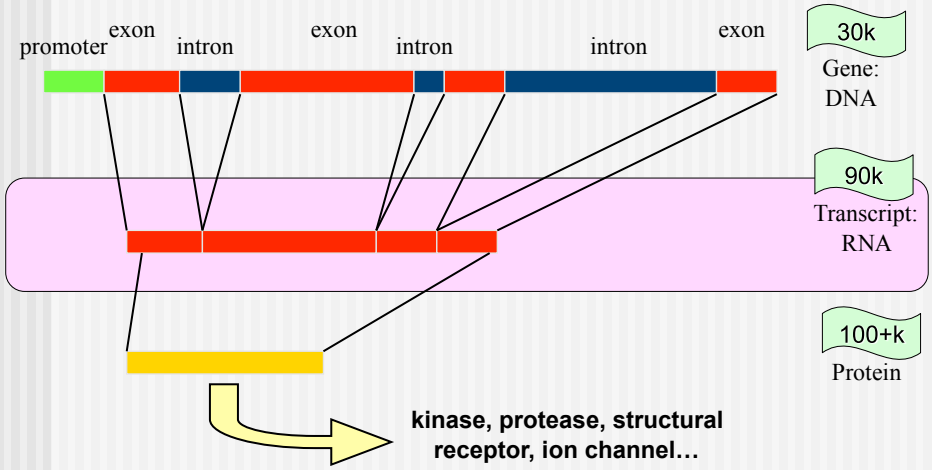
Microarray Informatics

Part 1

Biological research

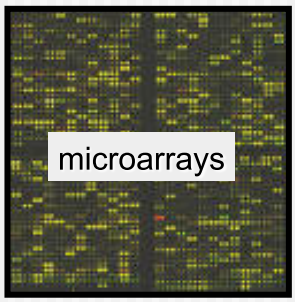
- Using a wide range of experimental and computational methods to answer biological questions
- Genetics, physiology, molecular biology…
- Biology and informatics → bioinformatics
- Genomic revolution
- What can we measure?

The central dogma



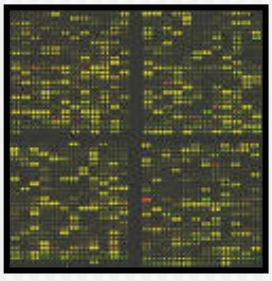
Measuring transcripts

- Genome level sequencing
- New miniaturisation technologies
- Better bioinformatics

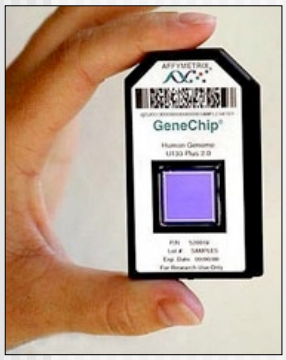


Microarrays: wish list

- Include all genes in the genome
- Include all splice variants
- Give reliable estimates of expression
- Easy to analyse
  - bioinformatics tools available
- Cost effective

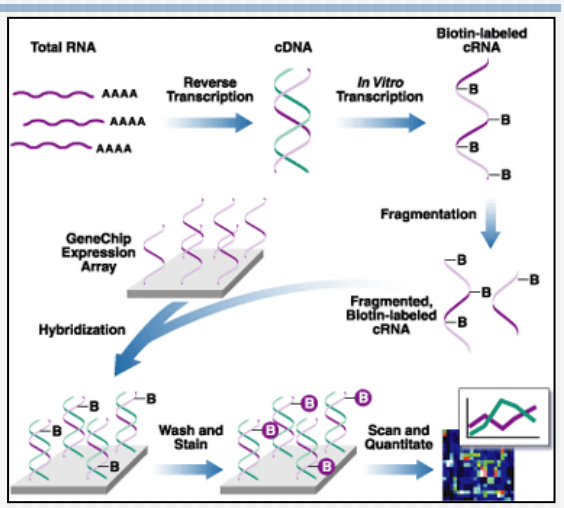


Microarray technologies - 1



- Oligonucleotides - Affymetrix
- One chip all genes
- Chips for many species
- Several oligos per transcript
- Use of control, mismatch sequences
- One sample per chip
  - ‘absolute quantification’
- Well established in research
- Expensive

Microarray technologies - 1



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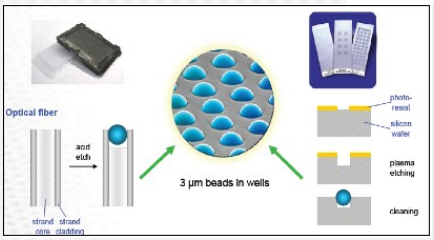
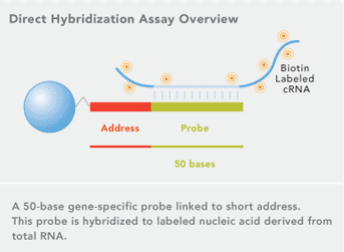
Microarray technologies - 2

- Illumina BeadChip
- Oligos on beads
- Hybridise in wells
- Compared to Affy
  - Higher throughput
  - Less RNA needed
  - Cheaper





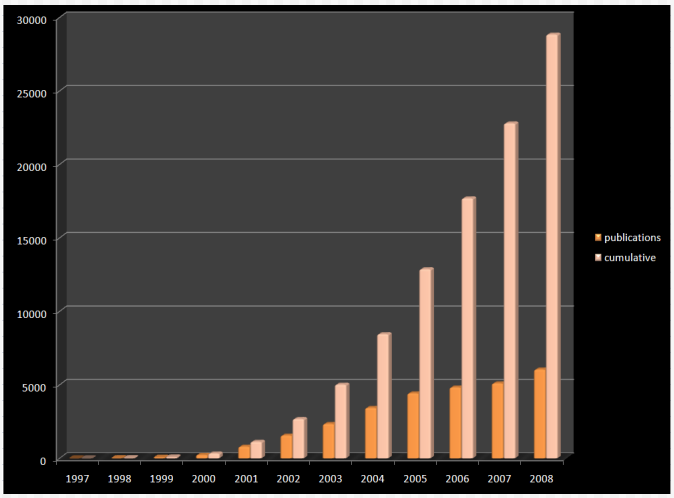
Problems with transcriptomics

- The gene might not be on the chip
- Can't differentiate splice variants
- The gene might be below detection limit
- Can't differentiate RNA synthesis and degradation
- Can't tell us about post translational events
- Bioinformatics can be difficult
- Relatively expensive

History of Microarrays

- Developed in early 1990s after larger macro-arrays (100-1000 genes)
- Microarrays were spotted on glass slides
- Labs spotted their own (Southern, Brown)
- Then companies started (Affymetrix, Agilent)
- Some early papers:
  - *Int J Immunopathol Pharmacol.* 1990 19(4):905-914. Raloxifene covalently bonded to titanium implants by interfacing with (3-aminopropyl)-triethoxysilane affects osteoblast-like cell gene expression. Bambini et al
  - *Nature* 1993 364(6437): 555-6 Multiplexed biochemical assays with biological chips. Fodor SP, et al
  - *Science* 1995 Oct 20;270(5235):467-70 Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Schena M, et al

Microarray publications



Types of experiment

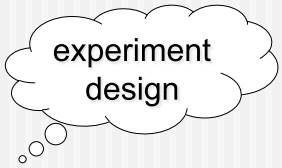
- Usually **control** v **test(s)**

Placebo	Drug treatment Drug 2…
Wild-type	Knockout
Healthy	Patient
Normal tissue	Cancerous tissue
Time = 0	Time = 1 Time = 2…

Types of experiment

- Usually **control v test(s)**
- But also **test v test(s)**
- Comparison:
  - placebo v drug treatment
  - drug 1 v drug 2
  - tissue 1 v tissue 2 v tissue 3 (pairwise)
  - time 0 v time 1, time 0 v time 2, time 0 v time 3
  - time 0 v time 1, time 1 v time 2, time 2 v time 3

A typical experiment

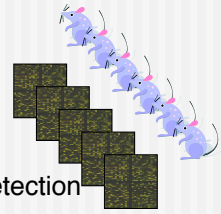


Experiment design: system

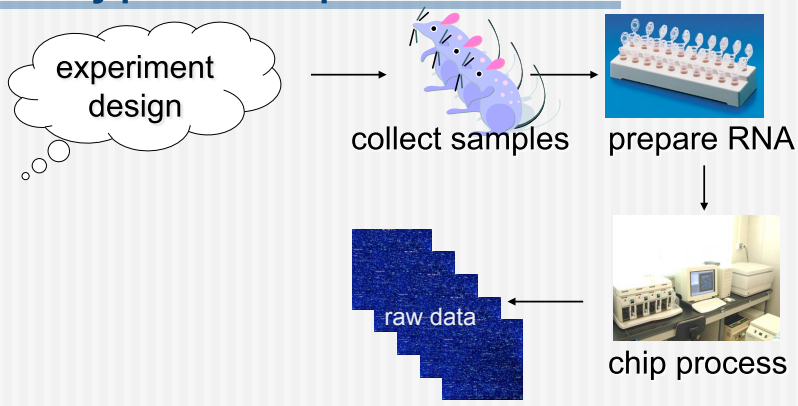
- What is your model?
  - animal, cell, tissue, drug, time…
- What comparison?
- What platform
  - microarray? oligo, cDNA?
- Record all information: see “standards”

Experiment design: replicates

- Microarrays are noisy: need extra confidence in the measurements
- We usually don't want to know about a specific individual
  - eg not an individual mouse, but the strain
  - although sometimes we do (eg people)
- Biological replicates needed
  - independent biological samples
  - number depends on variability and required detection
- Technical replicates (same sample, different chip) usually not needed



A typical experiment



Raw data

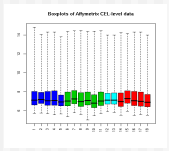
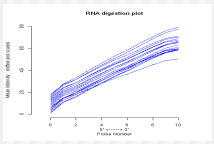
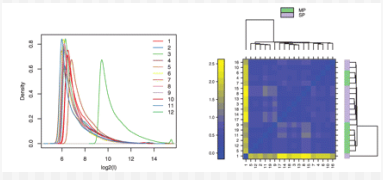
- Affymetrix GeneChip process generates:
  - DAT image file
  - CEL raw data file
  - CDF chip definition file
- Processing then involves CEL and CDF
- Will use Bioconductor

Bioconductor (BioC)

- http://www.bioconductor.org/
- "Bioconductor is an open source software project for the analysis and comprehension of genomic data"
- Started 2001, developed by expert volunteers
- Built on statistical programming environment "R"
- Provides a wide range of powerful statistical and graphical tools
- Use BioC for most microarray processing and analysis
- Most platforms now have BioC packages
- Make experiment design file and import data

Quality control (QC)

- Affymetrix gives data on QC
  - the microarray team will record these for you
  - scaling factor, % present, spiked probes, internal controls
- Bioconductor offers:
  - boxplots and histograms of raw and normalised data
  - RNA degradation plots
  - specialised quality control routines (eg arrayQualityMetrics)

Pre-processing: background

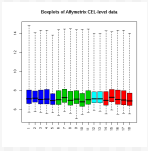
- Signal corresponds to expression…
  - plus a non-specific component (noise)
- Non specific binding of labelled target
- Need to exclude this background
- Several methods exist
  - eg Affy: PM-MM but many complications
  - eg RMA PM=B+S (don't use MM)

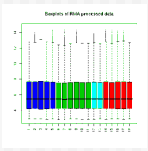
Pre-processing: normalisation

- In addition to background corrections
  - chip, probe, spatial, intra and inter [illegible]ation
  - need to remove to get at real ex[illegible] differences
- Make use of
  - [illegible]
- But seems to be no[illegible] dependency on intensity
  - additive and m[illegible]ive errors
- Quantile norm[illegible] often used
- Normalisati[illegible] complicated for 2-colour arrays
- Try to re[illegible]ost noise at lab stage (ie control things well statistically)

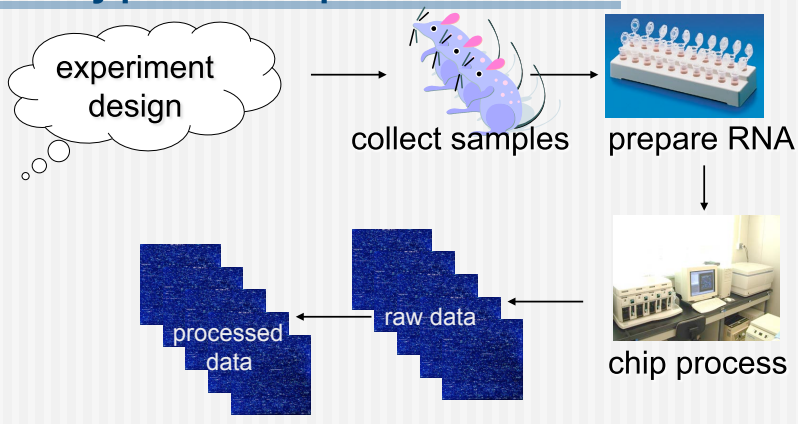
combined with [illegible]set summary:
get an expres[illegible]lue for the gene

Now carry on with analysis!





A typical experiment



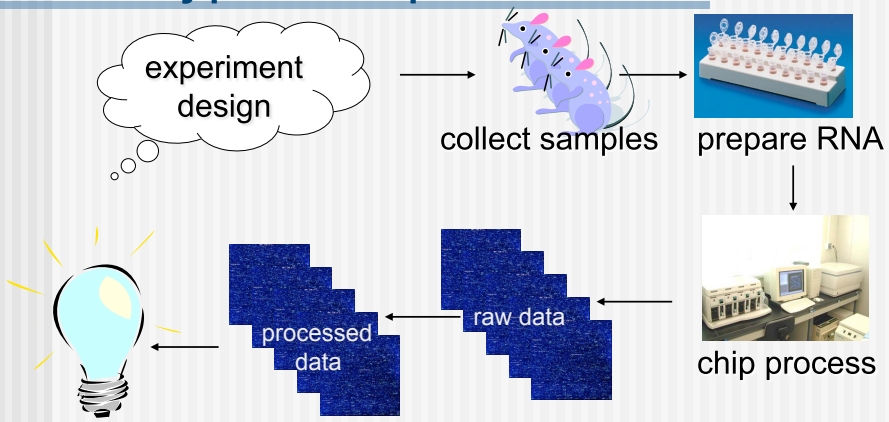
Part 1 Summary

- Microarrays in biological research
- Two types of microarray
- A typical microarray experiment
- Experiment design
- Data pre-processing

Microarray Informatics

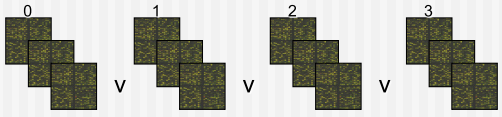
Part 2

A typical experiment



Data analysis

- Identifying differential expression
- Compare control and test(s)
  - t-test
  - ANOVA
  - SAM (FDR)
  - Limma
  - Rank Products
- Time series



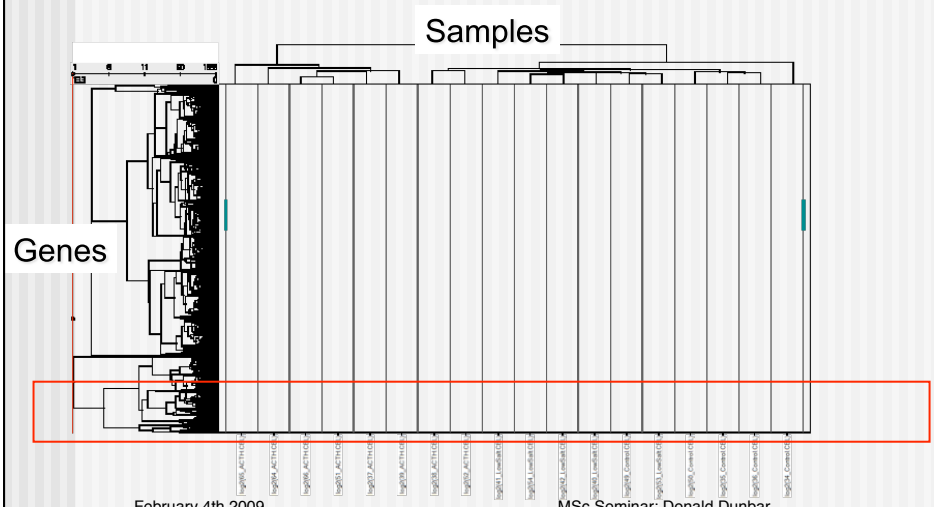
Multiple testing

- Problem:
  - statistical testing of 30,000 genes
  - at $\alpha = 0.05 \rightarrow$ 1500 genes
- Need to correct this
  - Multiply p-value by number of observations
    - Bonferroni, too conservative
  - False discovery
    - defines a q value: expected false positive rate
    - Less conservative, but higher chance of type I error
    - Benjamini and Hochberg
- Then regard genes as differentially expressed
- Depends on follow-up procedure!

Hierarchical clustering

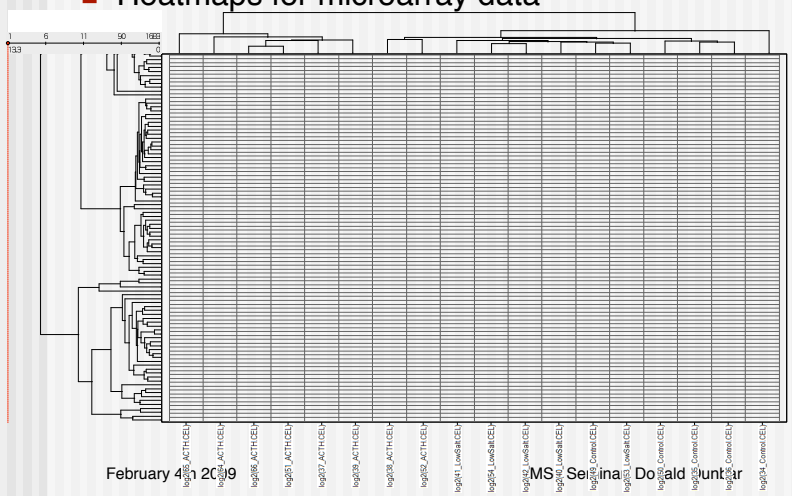
- Look for structure within dataset
  - similarities between genes
- Compare gene expression profiles
  - Euclidian distance
  - Correlation
  - Cosine correlation
- Calculate with distance matrix
- Combine closest, recalculate, combine closest… (or split!)
- Draw dendrogram and heatmap

Hierarchical clustering



Hierarchical clustering

- Heatmaps for microarray data



Hierarchical clustering

- Predicting association of known and novel genes
- Class discovery in samples: new subtypes
- Visualising structure in data (sample outliers)
- Classifying groups of genes
- Identifying trends and rhythms in gene expression
- Caveat: you will always see clusters, even when they are not particularly meaningful

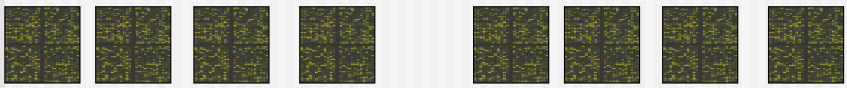
Sample classification

- Supervised or non-supervised
- Non-supervised
  - like hierarchical clustering of samples
- Supervised
  - have training (known) and test (unknown) datasets
  - use training sets to define robust classifier
  - apply to test set to classify new samples

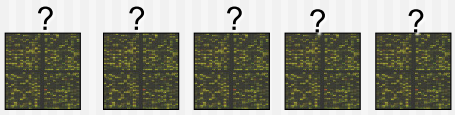
Sample classification

good prognosis
→ drug treatment

bad prognosis
→ surgery



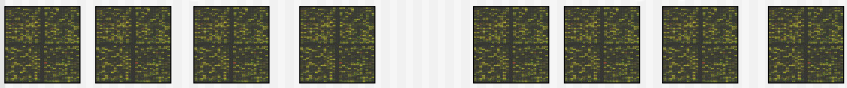
Gene selection, training, cross validation →
classifier: gene x * 0.5 gene y * 0.25 gene z …



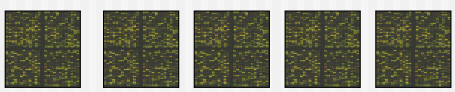
Sample classification

good prognosis
→ drug treatment

bad prognosis
→ surgery



Apply classifier



Sample classification

- Class prediction for new samples
  - cancer prognosis
  - pharmacogenomics (predict drug efficacy)
- Need to watch for overfitting
  - using too much of the data to classify
  - classifier loses specificity

Annotation

- Big problem for microarrays
- Genome-wide chips need genome-wide annotation
- Good bioinformatics essential
  - use several resources (Affymetrix, Ensembl)
  - keep up to date (as annotation changes)
  - genes have many attributes
    - name, symbol, gene ontology, pathway…

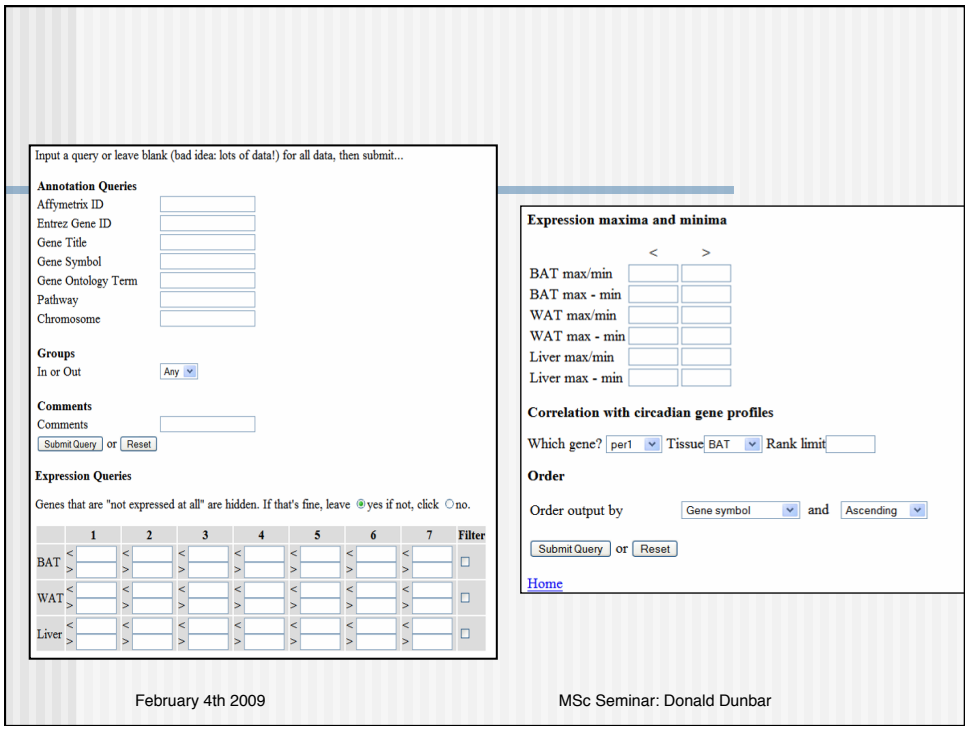
Data-mining

Microarrays are a waste of time

…unless you do something with the data

Data-mining

- Once data are statistically analysed:
  - pull out genes of interest
  - pull out pathways of interest
  - mine data based on annotation
    - what are the expression patterns of these genes
    - what are the expression patterns in this pathway
  - mine genes based on expression pattern
    - what types of genes are up-regulated …
    - fold change, p-value, expression level, correlation
- Should be driven by the biological question



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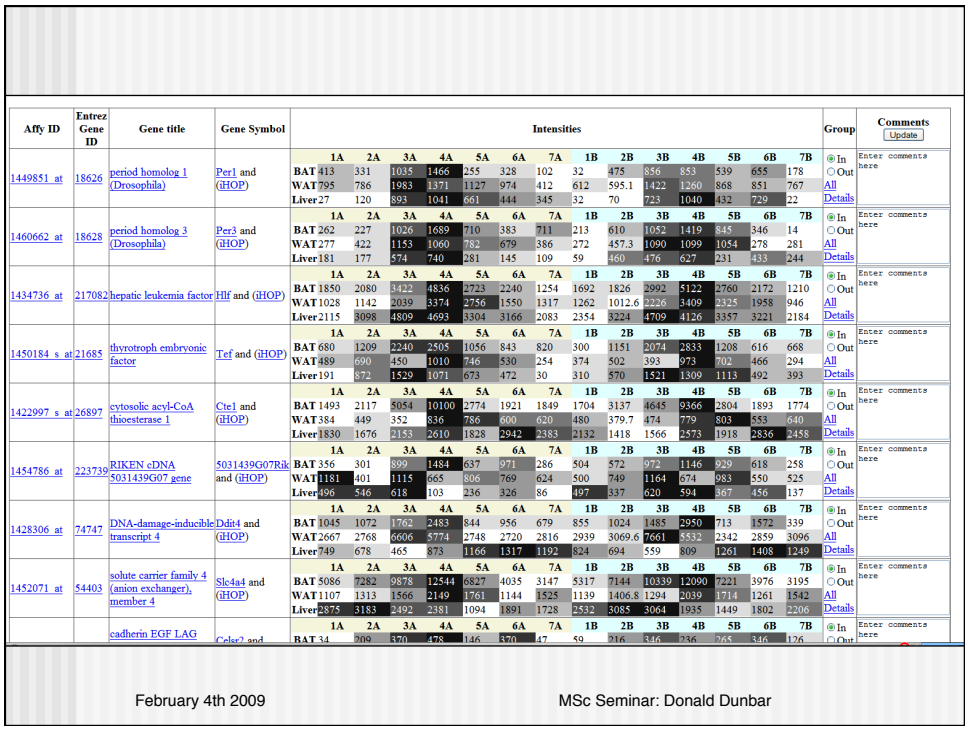

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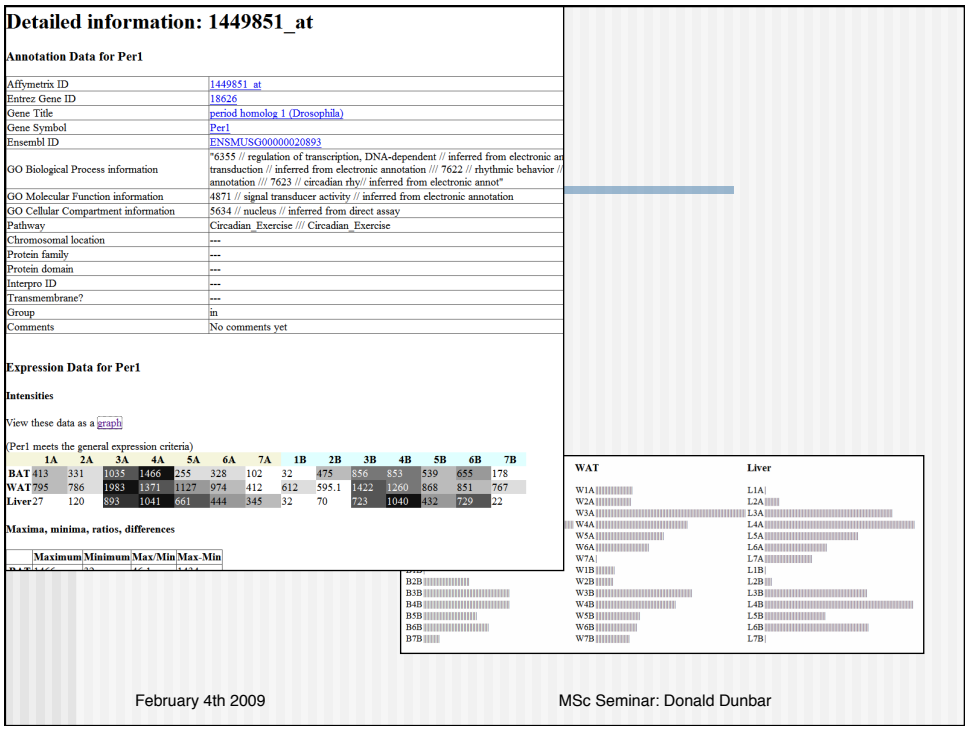
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Affymetrix ID 1449851_at
Entrez Gene ID 18626
Gene Title period homolog 1 (Drosophila)
Gene Symbol Per1
Ensembl ID ENSMUSG00000020893
GO Biological Process information "6355 // regulation of transcription, DNA-dependent // inferred from electronic an
transduction // inferred from electronic annotation /// 7622 // rhythmic behavior //
annotation /// 7623 // circadian rhy// inferred from electronic annot"
GO Molecular Function information 4871 // signal transducer activity // inferred from electronic annotation
GO Cellular Compartment information 5634 // nucleus // inferred from direct assay
Pathway Circadian_Exercise /// Circadian_Exercise
Chromosomal location ---
Protein family ---
Protein domain ---
Interpro ID ---
Transmembrane? ---
Group in
Comments No comments yet
Expression Data for Per1
Intensities
View these data as a graph
(Per1 meets the general expression criteria)
1A 2A 3A 4A 5A 6A 7A 1B 2B 3B 4B 5B 6B 7B
BAT 413 331 1035 1466 255 328 102 32 475 856 853 539 655 178
WAT 795 786 1983 1371 1127 974 412 612 595.1 1422 1260 868 851 767
Liver 27 120 893 1041 661 444 345 32 70 723 1040 432 729 22
Maxima, minima, ratios, differences
Maximum Minimum Max/Min Max-Min
WAT
Liver
W1A L1A
W2A L2A
W3A L3A
W4A L4A
W5A L5A
W6A L6A
W7A L7A
W1B L1B
W2B L2B
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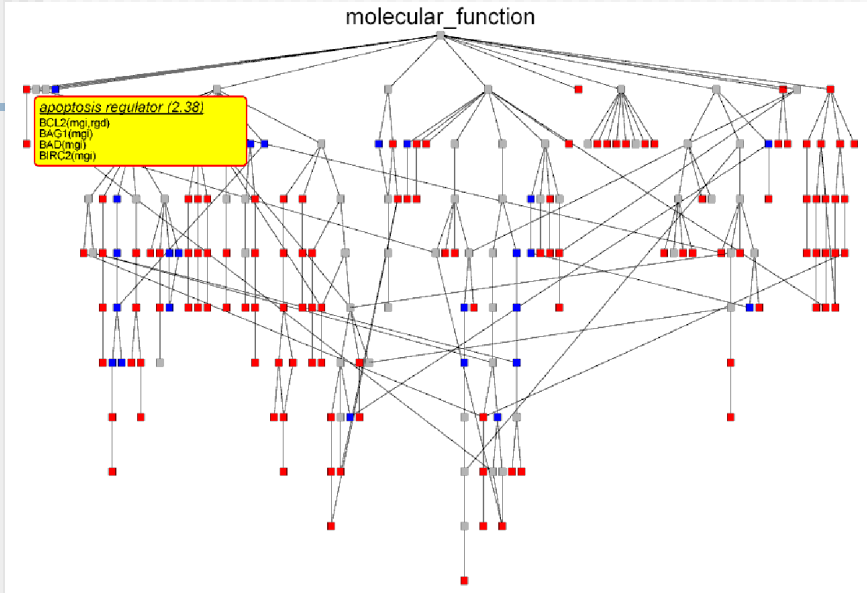
B6B W6B L6B
B7B W7B L7B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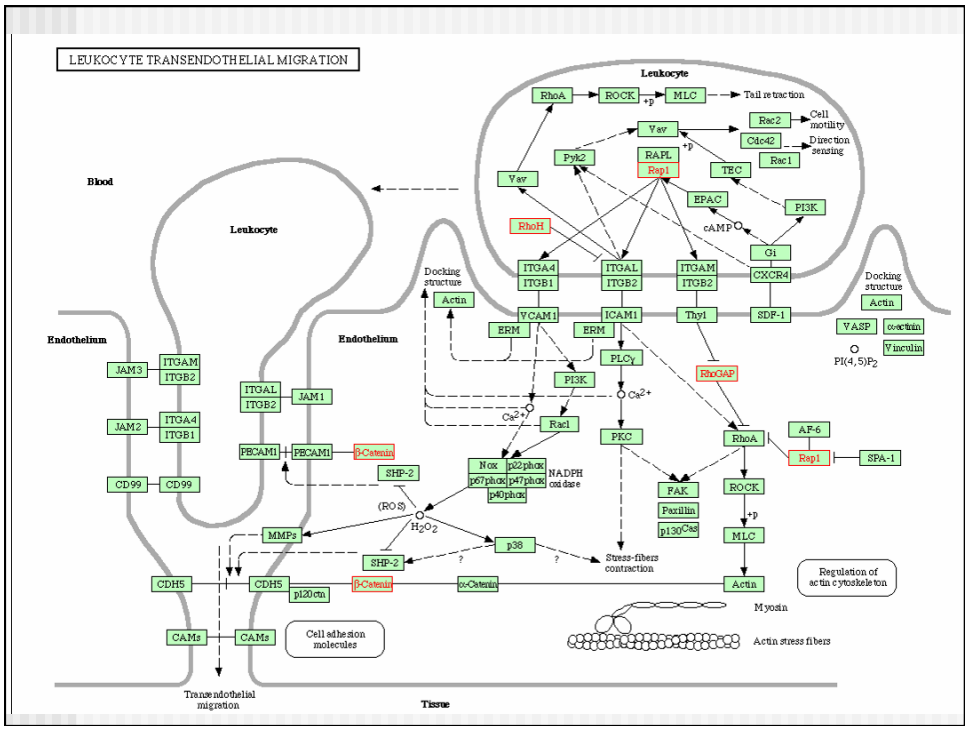
apoptosis regulator (2,38)
BCL2(mgi,rgd)
BAG1(mgi)
BAD(mgi)
BIRC2(mgi)


LEUKOCYTE TRANSENDOTHELIAL MIGRATION
Leukocyte
RhoA
ROCK
MLC
Tail retraction
Rac2
Cell motility
Vav
Cdc42
Direction sensing
Pyk2
RAPL
Rac1
Rap1
TEC
Vav
EPAC
PI3K
Blood
Leukocyte
RhoH
cAMP
Gi
ITGA4
ITGB1
ITGAL
ITGB2
ITGAM
ITGB2
CXCR4
Docking structure
Actin
VCAM1
ICAM1
Thy1
SDF-1
ERM
ERM
VASP
α-actinin
Endothelium
Endothelium
PLCγ
Vinculin
PI(4,5)P2
JAM3
ITGAM
ITGB2
PI3K
RhoGAP
ITGAL
ITGB2
JAM1
JAM2
ITGA4
ITGB1
Ca2+
Ca2+
Rac1
PKC
RhoA
AF-6
PECAM1
PECAM1
β-Catenin
Rap1
SPA-1
SHP-2
Nox
p22phox
p67phox
p47phox
p40phox
NADPH oxidase
CD99
CD99
PAK
ROCK
(ROS)
H2O2
Paxillin
+p
MMPs
p130Cas
MLC
p38
SHP-2
Stress-fibers contraction
CDH5
CDH5
β-Catenin
α-Catenin
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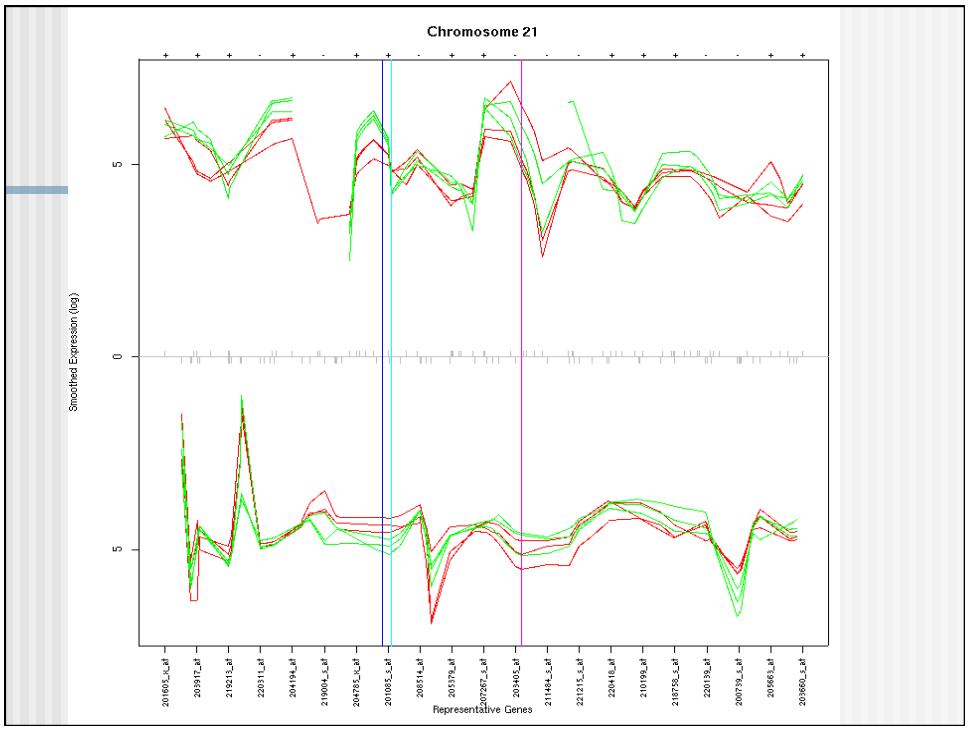
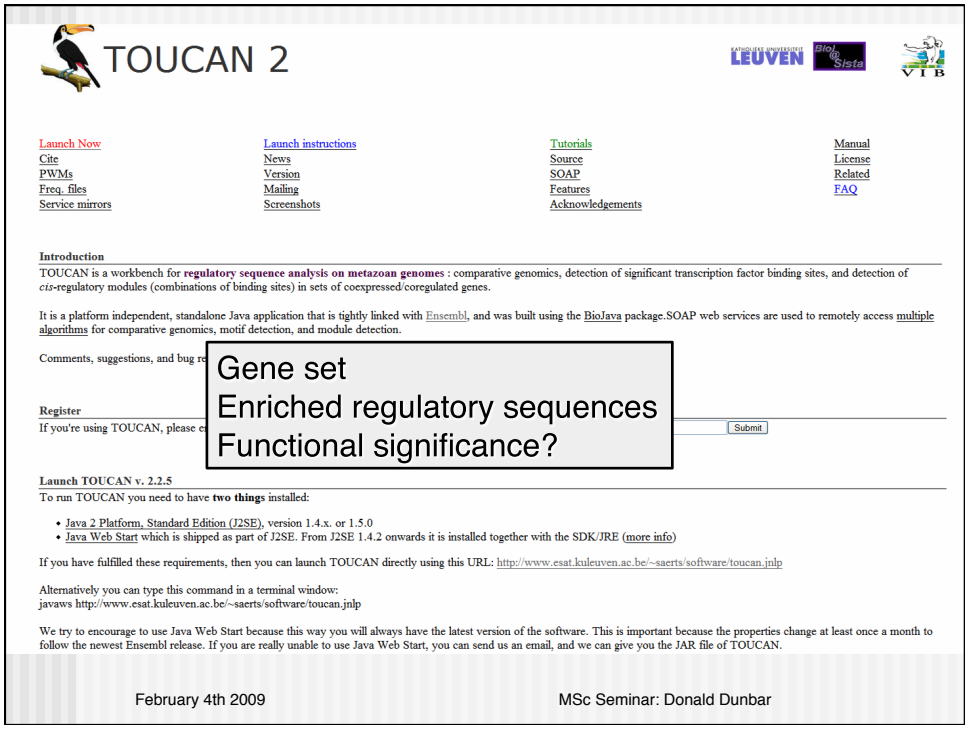
Regulation of actin cytoskeleton
p120ctn
Myosin
CAMs
CAMs
Cell adhesion molecules
Actin stress fibers
Transendothelial migration
Tissue

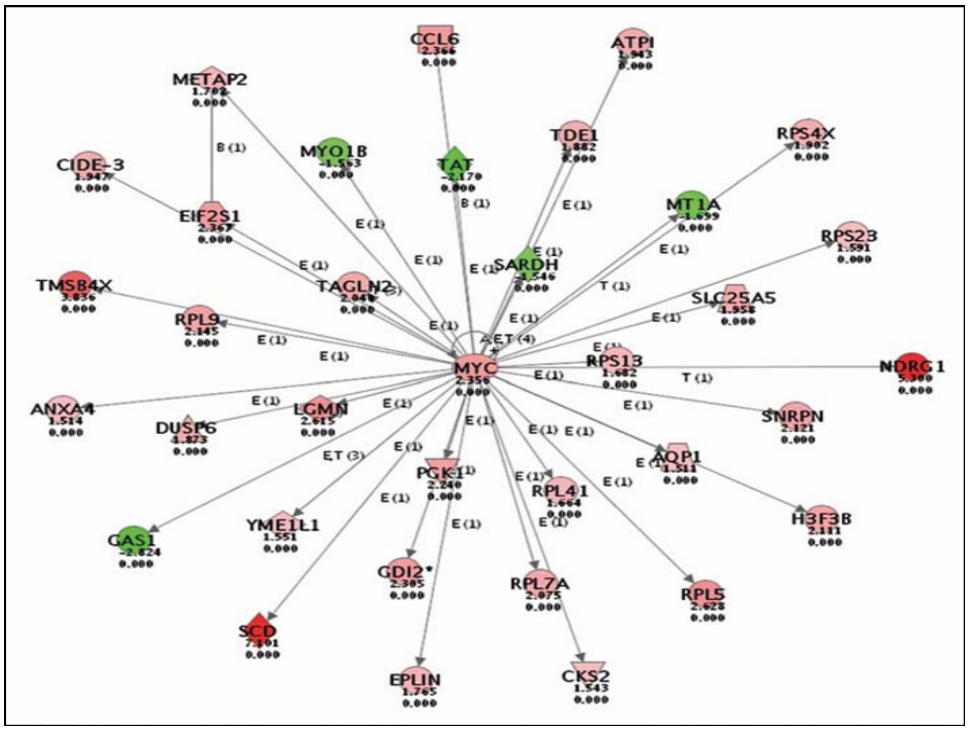

Chromosome 21
Smoothed Expression (log)
5
0
5
Representative Genes



PubMatrix Results 13th September 2005

Genes (ACTH differentially expressed) x Terms for DDUNBAR

PubMatrix	adrenal	hypertension	blood pressure	ACTH	low salt	low sodium	hyperplasia	hypoplasia	steroid	retention
(a disintegrin and metalloprotease domain 8 OR Adam8)	0	0	0	0	0	0	1	0	3	0
(abhydrolase domain containing 2 OR Abhd2)	0	0	0	0	0	0	1	0	0	0
(activating transcription factor 3 OR Atf3)	0	0	0	0	0	0	0	0	4	0
(acyl-CoA synthetase long-chain OR Acsl4)	13	4	1	2	0	0	1	6	20	1
(advillin OR Avil)	22	34	116	5	1	7	2	8	157	28
(alcohol dehydrogenase 7 OR Adh7)	3	3	7	1	1	6	1	8	51	6
(aldehyde dehydrogenase 1 family, member L2 OR Aldh1l2)	0	0	0	0	0	0	0	0	0	0
(aldehyde dehydrogenase 18 family, member A1 OR Aldh18a1)	0	0	0	0	0	0	0	0	0	0
(aldo-keto reductase family 1, member C18 OR Akr1c18)	0	0	0	0	0	0	0	0	1	0
(alkaline phosphatase 2, liver OR Akp2)	62	53	45	8	6	16	72	50	550	43
(arginine vasopressin-induced 1 OR Avpi1)	5	10	24	7	0	3	1	1	14	7
(bactericidal/permeability-increasing protein-like 2 OR Bpil2)	0	0	0	0	0	0	0	0	0	0
(basic leucine zipper and W2 domains 1 OR Bzw1)	0	0	0	0	0	0	0	0	0	0
(BMP-binding endothelial regulator OR MGI:1920480)	0	0	0	0	0	0	0	0	0	0
(branched chain aminotransferase 1, cytosolic OR Bcat1)	1	0	0	0	0	0	0	0	1	0
(CD5 antigen-like OR Cd5l)	0	0	0	0	0	0	0	0	0	0
(CDC28 protein kinase regulatory subunit 2 OR Cks2)	0	0	0	0	0	0	0	0	0	0
(CEA-related cell adhesion molecule 2 OR Ceacam2)	1	0	0	0	0	1	0	1	4	1
(cell division cycle 2 homolog OR Cdc2a)	0	0	0	0	0	0	0	1	1	1
(centaurin, gamma 2 OR Centg2)	0	0	0	0	0	0	0	0	0	0
(chitinase 3-like 3 OR Chi3l3)	0	0	0	0	0	0	0	0	0	0
(cyclin-dependent kinase inhibitor 1C OR Cdkn1c)	11	2	0	2	0	0	11	48	8	3
(cystatin 8 OR Cst8)	2	4	5	0	2	3	3	3	6	1
(cytochrome c oxidase OR Cox6a2)	104	41	106	14	17	78	24	76	409	49
(cytokine receptor-like factor 1 OR Crlf1)	0	1	0	0	0	0	0	0	0	0
(DNA (cytosine-5-)-methyltransferase 3-like OR Dnmt3l)	0	0	0	0	0	0	0	1	0	0
(down-regulated by Ctnnb1, a OR MGI:2149839)	0	0	0	0	0	0	0	0	0	0
(dystrobrevin alpha OR Dtna)	0	0	0	0	0	0	0	4	0	1
(embigin OR Emb)	1	5	3	1	0	1	3	5	23	0
(fatty acid desaturase 2 OR Fads2)	0	3	0	1	2	0	1	2	36	1
(frizzled-related protein OR Frzb)	1	0	0	0	0	0	1	1	12	0
(galactosidase, alpha OR Gla)	48	144	108	15	12	31	35	84	449	58



Further data-mining

- Other tools available using
  - gene ontology (GO)
  - biological pathways (eg KEGG)
  - genomic localisation (Ensembl)
  - regulatory sequence data (Toucan, BioProspector)
  - literature (eg Pubmatrix, Ingenuity…)
- … to make sense of the data

Microarray Resources

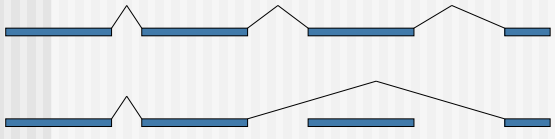
- Microarray data repositories
  - Array express (EBI, UK)
  - Gene Expression Omnibus (NCBI, USA)
  - CIBEX (Japan)
- Annotation
  - NetAffx, Ensembl, TIGR, Stanford…

Microarray Standards

- MIAME
  - Minimum annotation about a microarray experiment
  - Comprehensive description of experiment
  - Models experiments well, and allows replication
    - chips, samples, treatments, settings, comparisons
  - Required for most publications now
- MAGE-ML
  - Microarray gene expression markup language
  - Describes experiment (MIAME) and data
  - Tools available for processing

Recent advances: Exon chips

- Affymetrix now have chips that allow us to measure expression of splice variants



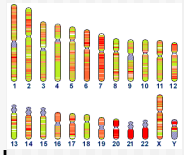
0.66 (down moderately)

1.4 (up slightly)

3 (up strongly)

New chips will give us much more information

Recent advances: Genotyping chips



- All discussion on EXPRESSION chips
- Also can get chips looking at genotype
- Tell us the sequence for genome-wide markers
- Test 300,000 markers with one chip
- Look for association with disease, prognosis, trait…
- Combined with expression chips to generate
  - EXPRESSION QUANTITATIVE TRAIT LOCUS (eQTL)
  - Overlap of expression and genetic differences (cis)
  - Correlation at different locus (trans)

Next Generation Sequencing

- Sequence rather than hybridisation
- Gene expression, genotyping, epigenetics
- New technologies: much cheaper than before
- Gene expression, genotyping, epigenetics
- Open ended (no previous knowledge required)
- Will take over in 5 years: the end of microarrays?

Part 2 Summary

- Data analysis
- Data Mining
- Microarray Resources
- Microarray Standards
- Recent advances

Seminar Summary

- Part 1
  - Microarrays in biological research
  - A typical microarray experiment
- Part 2
  - Data analysis and mining
  - Recent advances

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PhD opportunity

- Centre for Cardiovascular Science (Edinburgh)
- The Cellular and Molecular Basis of Cardiovascular Disease
- BHF funded PhDs
  - biologists (x4)
  - physical scientists (informatics, physics, maths….)
- Details on web:
  - http://www.cvs.med.ed.ac.uk/Training/content.asp?SubCatID=44