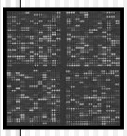
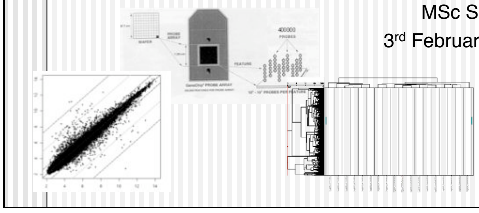


Microarray Informatics

Donald Dunbar

MSc Seminar

3rd February 2010



Aims

- To give a biologist's view of microarray experiments
- To explain some 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

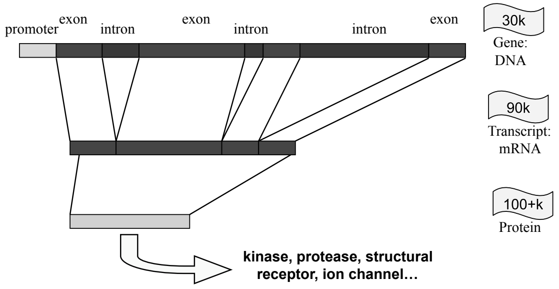
Microarray Informatics

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?

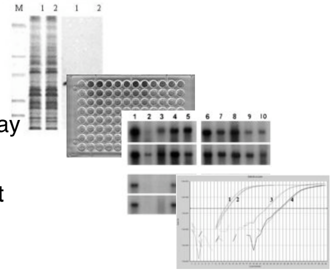
The central dogma



kinase, protease, structural receptor, ion channel…

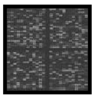
Measuring RNA and proteins

- Proteins
  - Western blot
  - ELISA
  - Enzyme assay
- mRNA
  - Northern blot
  - RT-PCR



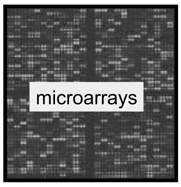
Measuring RNA and proteins

- Protein levels/activities would be best
  - no real high throughput method
- mRNA levels will have to do
  - genome-wide physical microarrays
  - other 'array-like' technologies
  - sequencing (see later)



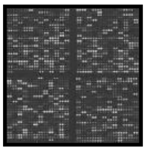
Measuring transcripts

- Genome level sequencing
- New miniaturisation technologies
- Better bioinformatics



Microarrays: wish list

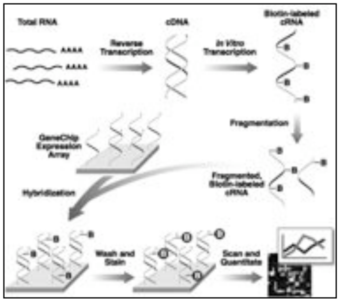
- Include all genes in the genome
- Include all splice variants
- Give reliable estimates of expression
- Easy to analyse
  - bioinformatics tools available
- Cost effective



Microarray technologies - 1

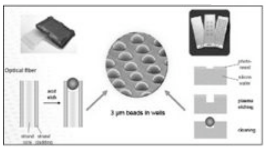
- Oligonucleotides - Affymetrix
- One chip all genes
- Chips for many species
- Several oligos per transcript
- Use of control, mismatch sequences
- One sample per chip
  - 'absolute quantification'
- Well established in research
- Expensive

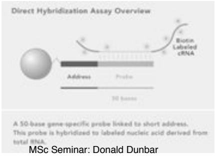
Microarray technologies - 1



Microarray technologies - 2

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





A 50-base gene-specific probe linked to short address. This probe is hybridized to labeled nucleic acid derived from total RNA.

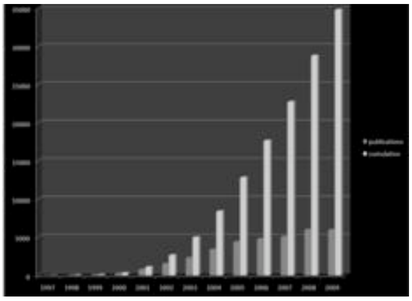
Problems with microarrays

- 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:
  - *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

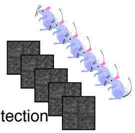
experiment design

Experiment design: system

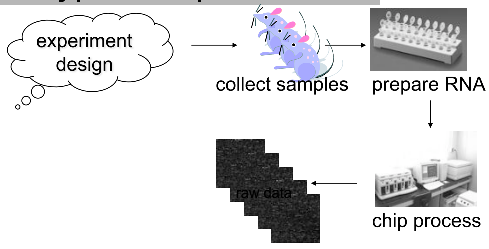
- What is your model?
  - animal, cell, tissue, drug, time…
- What comparison?
- What platform
  - microarray? oligo, cDNA?
- Record all information: see “standards”

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



experiment design

collect samples

prepare RNA

chip process

raw data

Raw data

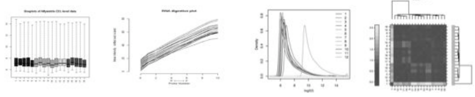
- Affymetrix GeneChip process generates:
  - DAT image file
  - CEL raw data file
  - CDF chip definition file
- Processing then involves CEL and CDF
- Will use Bioconductor

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
- Tutorial: manuals.bioinformatics.ucr.edu/home/R_BioCondManual

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)

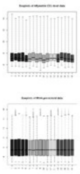
Pre-processing: normalisation

- In addition to background corrections[illegible]
  - chip, probe, spatial, intra and inter-[illegible]ation
  - need to remove to get at real ex[illegible] differences
- Make use of
  - statistics

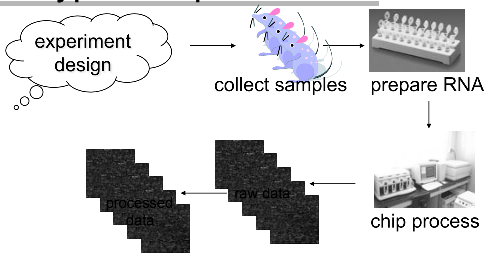
combined with [illegible]set summary:
get an expres[illegible]alue for the gene

- But seems to be no[illegible] dependency on intensity
  - additive and m[illegible]ive errors
- Quantile norm[illegible]n often used
- Normalisati[illegible]e complicated for 2-colour arrays
- Try to re[illegible]ost noise at lab stage (ie control things well statistical[illegible]

Now carry on with analysis!



A typical experiment



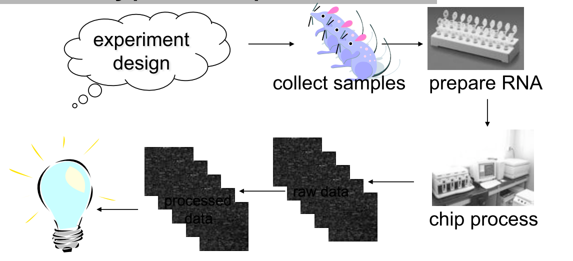
Part 1 Summary

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

Microarray Informatics

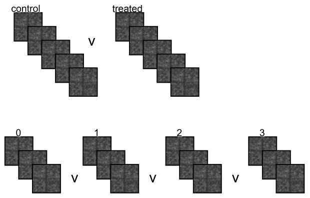
Part 2

A typical experiment



Data analysis

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



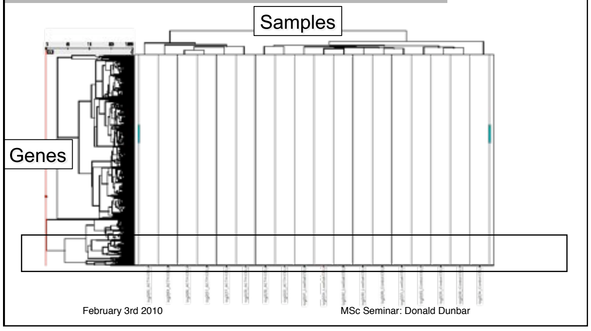
Multiple testing

- Problem:
  - statistical testing of 30,000 genes
  - at $\alpha = 0.05$ → 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

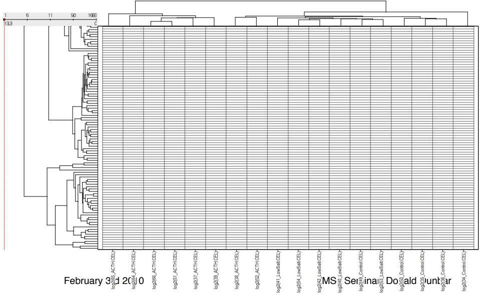
- Look for structure within dataset
  - similarities between genes
- Compare gene expression profiles
  - Euclidian distance
  - Correlation
  - Cosine correlation
- Calculate with distance matrix
- Combine closest, recalculate, combine closest… (or split!)
- Draw dendrogram and heatmap

Hierarchical clustering



Hierarchical clustering

- Heatmaps for microarray data



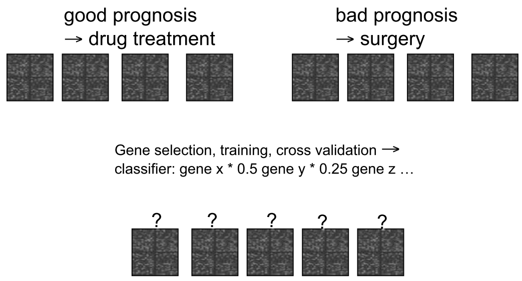
Hierarchical clustering

- 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 (nb Ian Simpson)

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

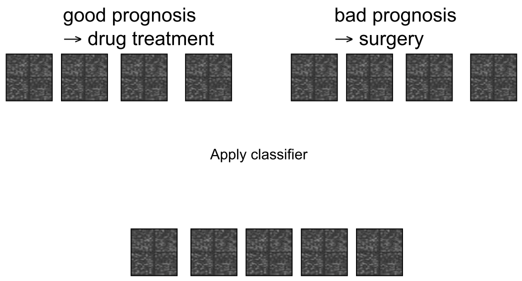


good prognosis
→ drug treatment

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…

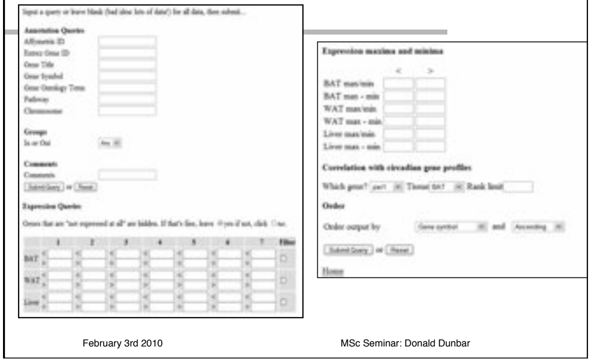
Data-mining

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



Input a query or leave blank (bad idea: lots of data!) for all data, then submit...

Annotation Queries

Affymetrix ID
Entrez Gene ID
Gene Title
Gene Symbol
Gene Ontology Term
Pathway
Chromosome

Groups

In or Out: Any

Comments

Comments

Submit Query or Reset

Expression Queries

Genes that are "not expressed at all" are hidden. If that's fine, leave ○ yes if not, click ○ no

	1	2	3	4	5	6	7	Filter
BAT	< >	< >	< >	< >	< >	< >	< >	□
WAT	< >	< >	< >	< >	< >	< >	< >	□
Liver	< >	< >	< >	< >	< >	< >	< >	□

Expression maxima and minima

	<	>
BAT max/min		
BAT max - min		
WAT max/min		
WAT max - min		
Liver max/min		
Liver max - min		

Correlation with circadian gene profiles

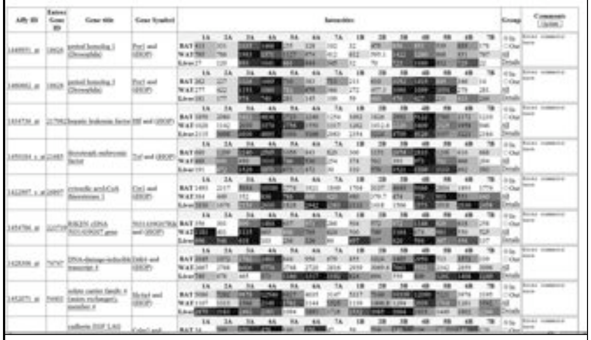
Which gene? per1 Tissue BAT Rank limit

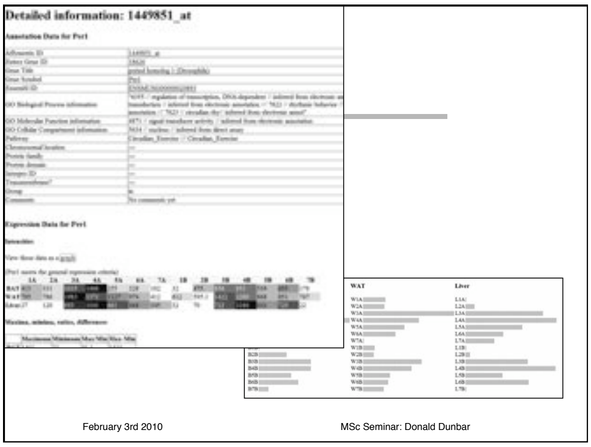
Order

Order output by Gene symbol and Ascending

Submit Query or Reset

Home





Detailed information: 1449851_at

Annotation Data for Per1

Affymetrix ID	1449851_at
Entrez Gene ID	18626
Gene Title	period homolog 1 (Drosophila)
Gene Symbol	Per1
Ensembl ID	[illegible]
GO Biological Process information	[illegible]
GO Molecular Function information	[illegible]
GO Cellular Component information	[illegible]
Pathway	Circadian_Exercise // Circadian_Exercise
Chromosomal location	---
Protein family	---
Protein domain	---
Interpro ID	---
Transmembrane?	---
Group	in
Comments	No comments yet

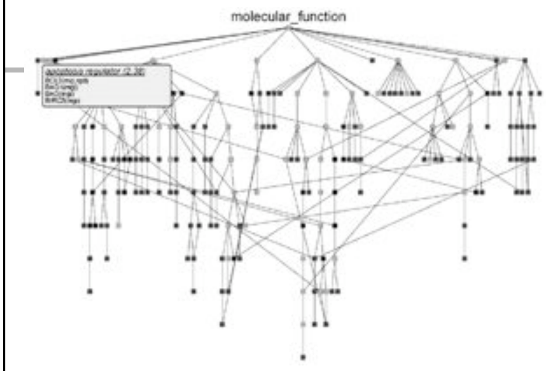
Expression Data for Per1

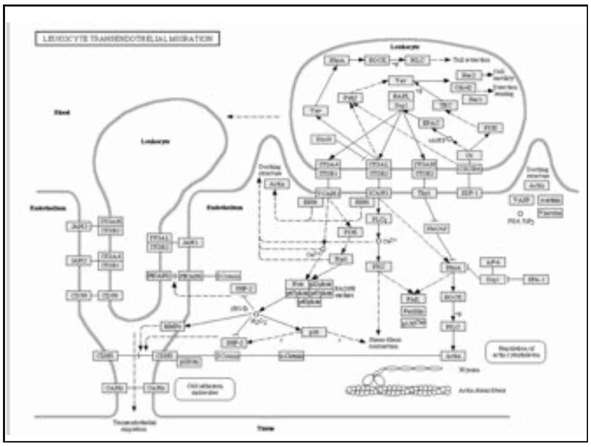
Intensities

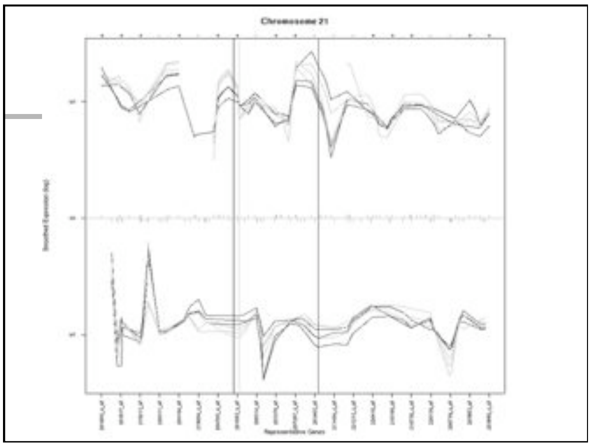
View these data as a graph

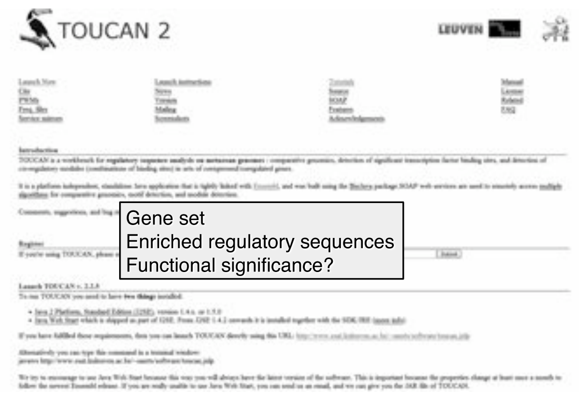
(Per1 meets the general expression criteria)

Maxima, minima, ratios, differences









Introduction

TOUCAN is a workbench for regulatory sequence analysis on metazoan genomes : comparative genomics, detection of significant transcription factor binding sites, and detection of co-regulatory modules (combinations of binding sites) in sets of coexpressed/coregulated genes.

It is a platform independent, standalone Java application that is tightly linked with Ensembl, and was built using the BioJava package. SOAP web services are used to remotely access multiple algorithms for comparative genomics, motif detection, and module detection.

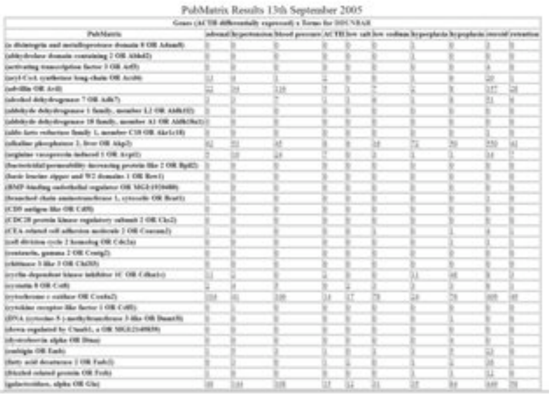
Register

Launch TOUCAN v. 2.2.8

To run TOUCAN you need to have two things installed:

- Java 2 Platform, Standard Edition (J2SE), version 1.4.x, or 1.5.0
- Java Web Start which is shipped as part of J2SE. From J2SE 1.4.2 onwards it is installed together with the SDK/JRE (more info)

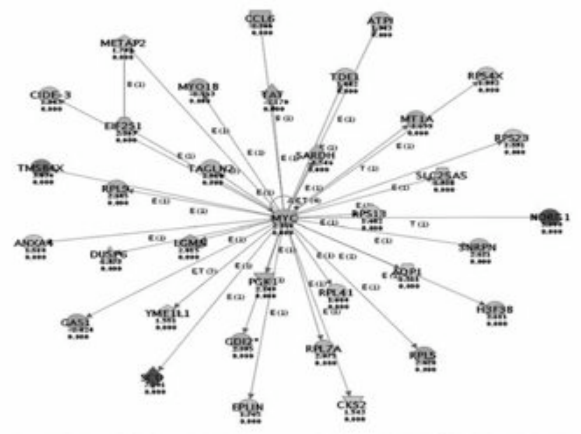
Gene set
Enriched regulatory sequences
Functional significance?



PubMatrix Results 13th September 2005

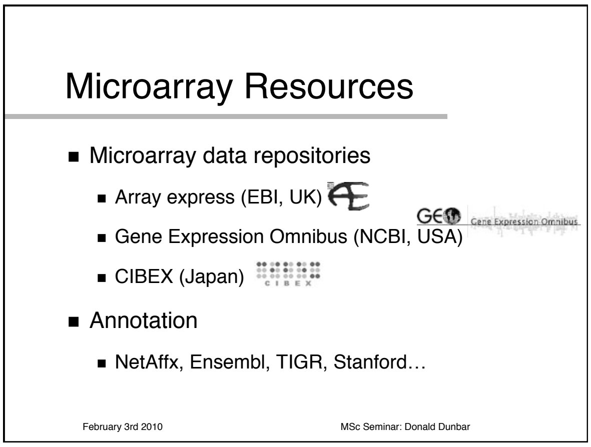
Genes (ACTH differentially expressed) x Terms for DDUNBAR

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	2	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	0	20	1
(advillin OR Avil)	22	39	118	7	3	7	2	3	157	28
(alcohol dehydrogenase 7 OR Adh7)	2	2	7	1	1	4	1	1	51	4
(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	8	10	71	20	250	41
(arginine vasopressin-induced 1 OR Avpi1)	7	10	23	7	0	2	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	2	0
(CD9 antigen-like OR Cd9l)	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	3	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	2
(cystatin 8 OR Cst8)	2	4	3	0	2	2	2	1	8	1
(cytochrome c oxidase OR Coxfa2)	334	41	108	14	17	78	24	71	405	48
(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)	1	0	0	0	0	0	0	1	0	0
(down-regulated by Ctnnb1, a OR MGI:2140839)	0	0	0	0	0	0	0	0	0	0
(dystrobrevin alpha OR Dtna)	0	0	0	0	0	0	0	0	0	1
(endoglin OR Eng)	1	0	2	1	0	1	1	7	22	0
(fatty acid desaturase 2 OR Fads2)	0	2	0	1	1	1	1	2	38	1
(frizzled-related protein OR Frzb)	1	0	0	0	0	0	1	1	12	0
(galactosidase, alpha OR Gla)	40	144	108	27	12	21	27	84	169	29



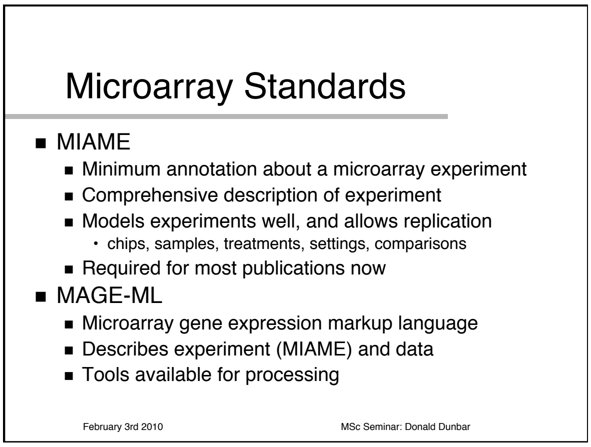
Further data-mining

- 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
- Links at: www.bioinf.mvm.ed.ac.uk/projects/analysis_tools.html



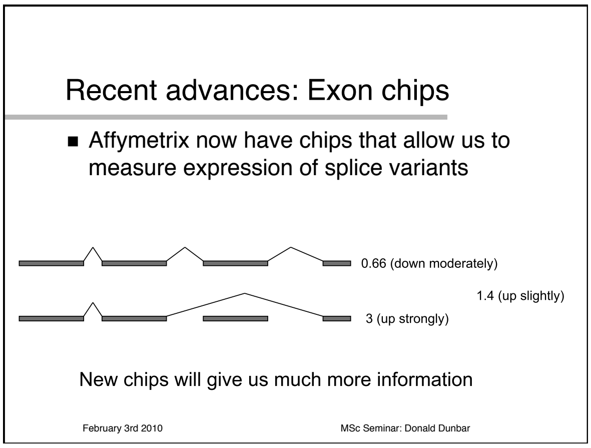
Microarray Resources

- 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

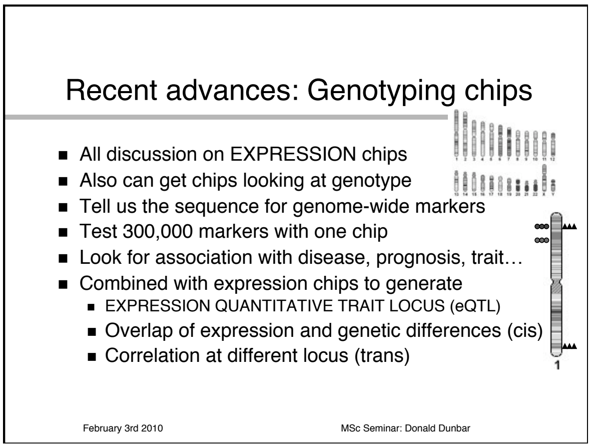
- Affymetrix now have chips that allow us to measure expression of splice variants

0.66 (down moderately)

1.4 (up slightly)

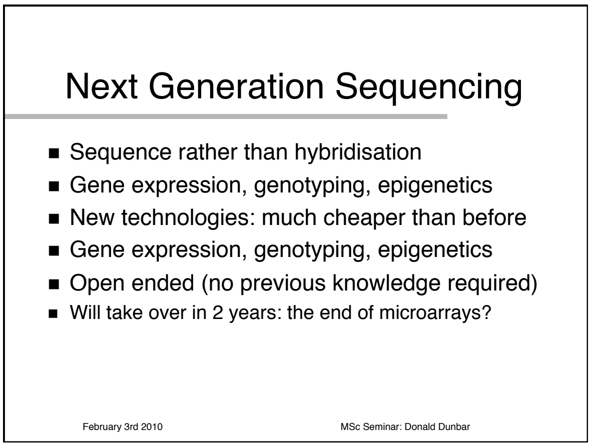
3 (up strongly)

New chips will give us much more information



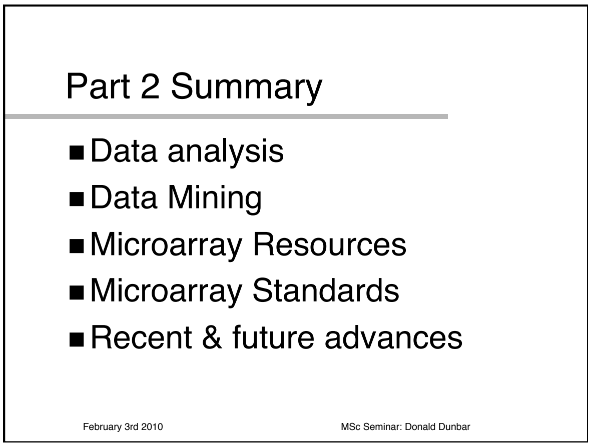
Recent advances: Genotyping chips

- 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 2 years: the end of microarrays?



Part 2 Summary

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

Seminar Summary

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

Contact

- Donald Dunbar
- QMRI Bioinformatics
- donald.dunbar@ed.ac.uk
- 0131 242 6700
- Room W3.01, QMRI, Little France
- www.bioinf.mvm.ed.ac.uk