BIOMENG 261

TISSUE AND BIOMOLECULAR ENGINEERING

Module I: Reaction kinetics and systems biology

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LECTURE 12: GENE EXPRESSION DATA AND GRNS

- *Larger systems*
  - Gene space and gene regulatory networks (GRNs)
- *Brief overview of microarray data*
  - Experiment types
  - Data organisation and expression matrices
- *Analysis types*
  - Clustering, distance matrices and dendrograms
  - Control analysis and regulatory matrices

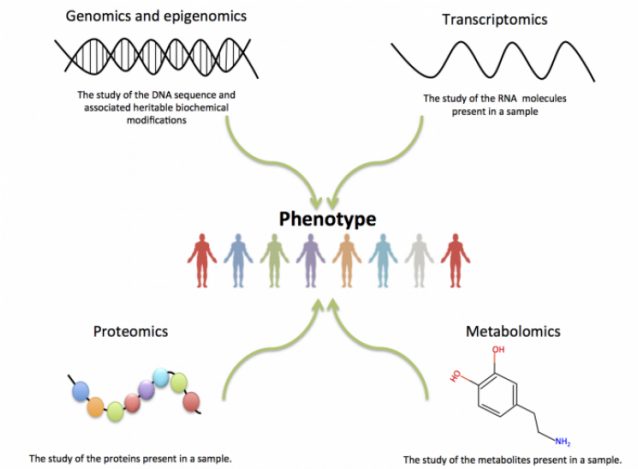
Note: there are many images stolen from the internet in what follows…

MODULE OVERVIEW

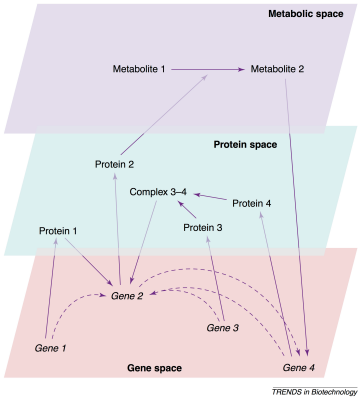
Reaction kinetics and systems biology (*Oliver Maclaren*)
[12 lectures/4 tutorials/2 labs]

1. *Basic principles: modelling with reaction kinetics* [6 lectures]
   Physical principles: conservation, directional and constitutive. Reaction modelling. Mass action. Enzyme kinetics. Enzyme regulation. Mathematical/graphical tools for analysis and fitting.
2. *Systems biology I: overview, signalling and metabolic systems* [3 lectures]
   Overview of systems biology. Modelling signalling systems using reaction kinetics. Introduction to parameter estimation. Modelling metabolic systems using reaction kinetics. Flux balance analysis and constraint-based methods.
3. *Systems biology II: genetic systems* [3 lectures]
   Modelling genes and gene regulation using reaction kinetics. Gene regulatory networks, transcriptomics and analysis of microarray data.

MUCH LARGER SYSTEMS - 'OMICS'



GENE SPACE



See: Brazhnik et al. (2002) 'Gene networks - how to put the function in genomics' (on Canvas)

EXPRESSION ANALYSIS

- *Microarrays*
- Mature technology
- Relatively well-established data analysis methods
- *RNA-seq*
- Newer technology, rapidly overtaking microarrays
- Less standardisation of analysis methods
- Much more computationally/storage intensive

But: *microarrays still relevant and useful*: we will consider these (easier and better understood)

TRANSCRIPTOMICS

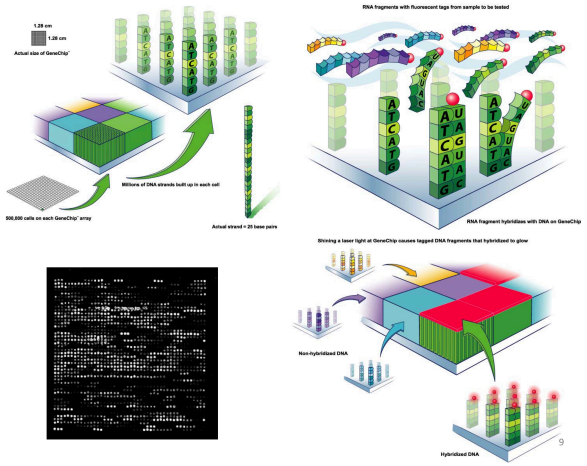
- A subfield of *functional genomics*
- Functional genomics: study of how genes and intergenic regions contribute to biological function
- The focus is on *gene expression*
- In particular, via *measuring mRNA* (the transcripts)

See: Lowe et al. (2017) 'Transcriptomics technologies' (on Canvas)

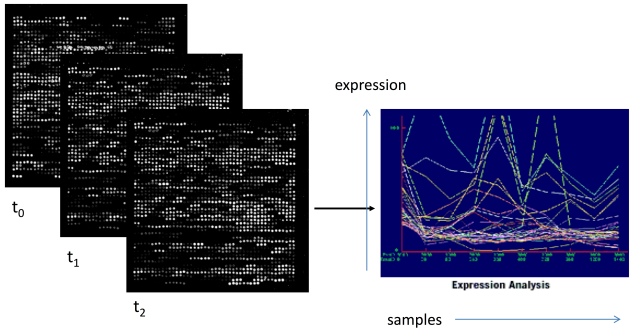
MICROARRAYS

For video intros: see e.g. https://youtu.be/0ATUjAxNf6U or https://youtu.be/VNsThMNjKhM

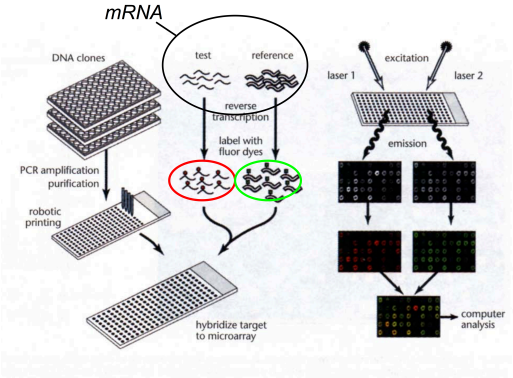
MICROARRAYS

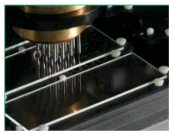


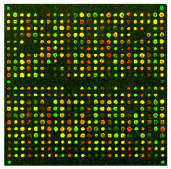
MICROARRAYS: TIME SERIES



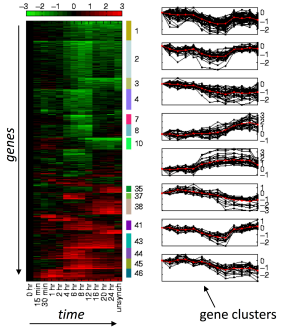
MICROARRAYS: COMPARATIVE EXPRESSION







MICROARRAYS: RELATIVE EXPRESSION OVER TIME



green – *down* regulation
red – *up* regulation

DATA ANALYSIS: STATISTICAL/MACHINE LEARNING

Clustering, unsupervised and supervised learning etc. see:

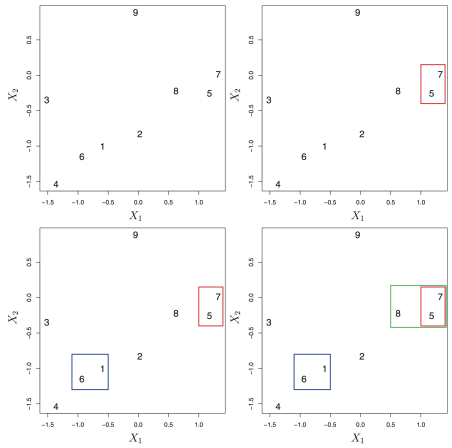
- James et al. 'An Introduction to Statistical Learning'
  - Available at: http://www-bcf.usc.edu/~gareth/ISL/
- Hastie et. al 'Elements of Statistical Learning: Data Mining, Inference and Prediction'
  - Available at: http://web.stanford.edu/~hastie/ElemStatLearn/

CLUSTERING

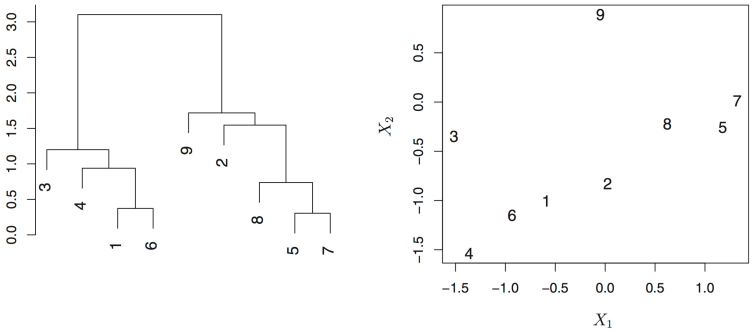
- An *unsupervised learning* method for *pattern discovery*
- Two popular algorithms are
  - K-means
  - Hierarchical clustering

See James et al. Chapter 10 for detailed algorithms. We will look at *hierarchical clustering* here.

HIERARCHICAL CLUSTERING EXAMPLE (JAMES ET AL.)



HIERARCHICAL CLUSTERING: DENDROGRAMS



PERTURBATION APPROACH FOR INFERRING REGULATORY MATRICES/NETWORKS

- Perturb *transcription rates* for *each gene in turn*
- Measure changes in *steady-state expression levels* for all genes (including self)
- Gives indication of underlying *regulatory network*
- Summarise in *regulatory strength matrix or network diagram*.

See de la Fuente et al. (2002) 'Linking the genes' (on Canvas)

EXAMPLE PAST QUESTIONS

(b) DNA microarrays are used are used to simultaneously measure the expression of many different genes in a sample. Explain briefly the difference between *time series* and *comparative* microarray measurements.

(4 marks)

(c) In a series of experiments, the amounts of mRNA for different genes are perturbed and the changes in mRNA of all genes of interest (namely A, B and C) are measured. A set of 'co-control coefficients' was calculated and organised into the 'Regulatory Strength' matrix, *Rd*, given below

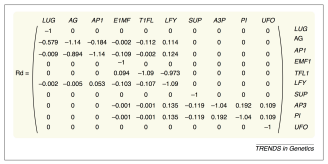
$$Rd = \begin{array}{c} \begin{matrix} A & B & C \end{matrix} \\ \begin{bmatrix} -0.5 & 0 & 0 \\ 0.1 & 0 & 1.2 \\ 0.8 & -0.1 & 1 \end{bmatrix} \end{array}$$

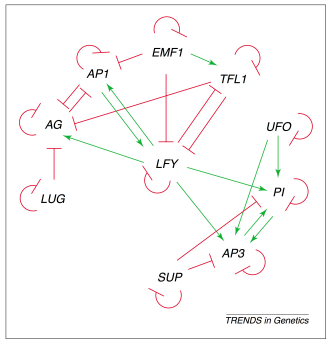
Using the information in *Rd* sketch a qualitative regulatory network showing how each gene regulates the expression of all genes (including itself). Use arrows (→) to show positive regulation and blunt arrows (⊣) to indicate negative regulation.

(5 marks)

PERTURBATION APPROACH FOR INFERRING REGULATORY MATRICES/NETWORKS

Example: flower morphogenesis (see de la Fuente et al. 2002 for details):

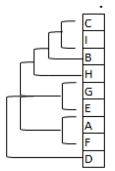




EXAMPLE PAST QUESTIONS

5)

a) In a microarray experiment, the expression of 8 genes was measured as a function of time and the data were analysed to create the following dendrogram:



State if the following statements are TRUE, FALSE or INDETERMINATE.

i) Genes C and I show similar expression patterns at the different time points.

(1 mark)

ii) The Euclidean distance between genes C and I is less than the Euclidean distance between genes I and B.

(1 mark)

Bioeng 261 Lecture 12

- Large genetic systems
  - 'Gene space' & GRNs (genetic regulatory networks)
  - Expression analysis methods

~~Takeaways~~ Upshot: know/interpret/draw

- matrix types
  - ↳ Gene expression matrix } main general format
    - ↳ gives { distance matrix ① for clustering
      regulatory strength matrix ② for control analysis
- dendrograms
  - ↳ summarise distance matrix / clustering algorithm ①
- regulatory networks
  - ↳ summarise regulatory strength matrix ② (control analysis).

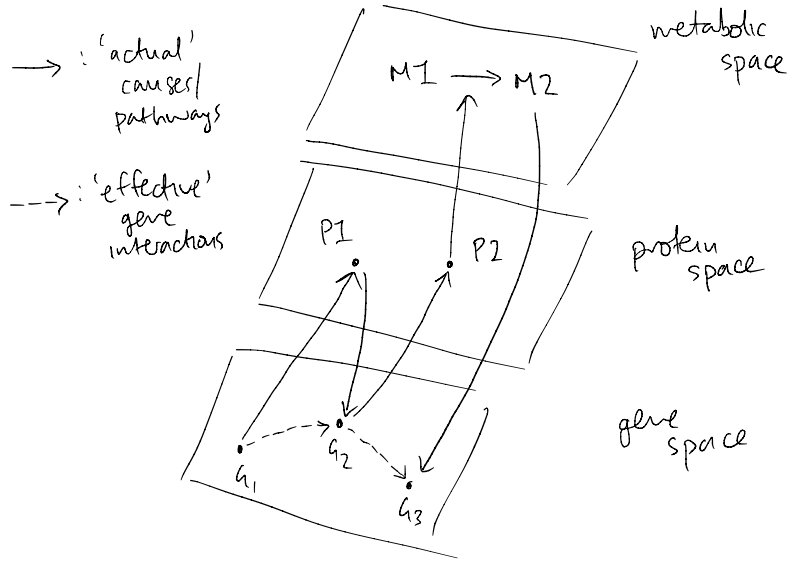
Setting: even more complex networks than last time

→ eg 1000s of genes (or more!)

(Background):

Gene Space

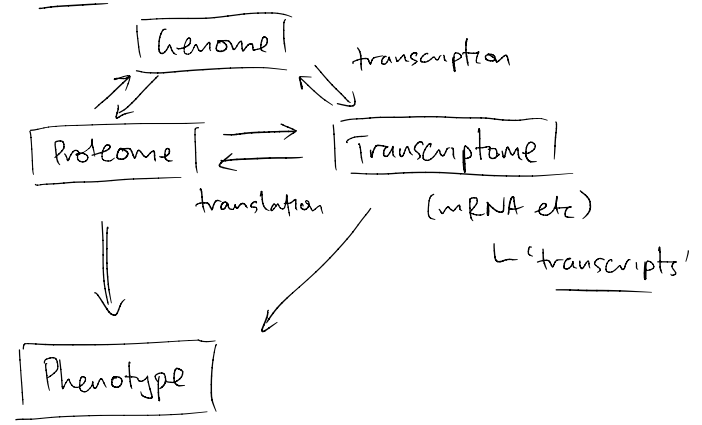
- A way of 'projecting all the action' down into interactions between genes



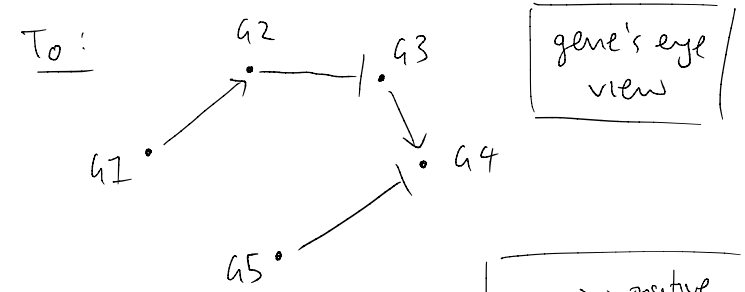
(Background)

Gene Regulatory Networks

From:



To:


G2
G3
G1
G4
G5

gene's eye view

ie a Gene Regulatory Network (GRN)

→ : positive
⊣ : negative

Transcriptomics

- A subfield of 'functional genomics'
  - ie the study of how genes & intergenic (between gene) regions contribute to biological function

- Transcriptomics focuses on gene expression levels
  - in particular as measured via the levels of the transcripts (mRNA) associated with genes
    - → mRNA easier to measure etc that proteins, but see 'proteomics'

Ideas:
- does expression go up or down 'under treatment'?
- do groups of genes go up/down together?

Expression Analysis

Two key approaches:

- **Microarrays**
  - uses (known) 'probes' (eg cDNA)
  - samples 'hybridise' if complementary to probes
  - amplify & quantify via qPCR
    - PCR: polymerase chain reaction (see lab lectures)

  ] need to know roughly what looking for

- **RNA-seq**
  - direct sequencing of transcripts
  - 'next gen', high-throughput sequencing

  ] don't need pre-chosen 'probes'

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* we will discuss **microarrays** *
  - well understood
  - more mature & easier to analyse
  - still used & useful
  - ... but RNA-seq overtaking!

(see Lowe et al. 2017 'Transcriptomics technologies')

Microarrays: (more) background (see slides & videos etc)

Idea: want to measure mRNA levels (hence gene expression)

- Microarrays can measure **1000s** of mRNA levels at a time
  - ↳ ie **1000s of genes at a time**

→ consist of a grid of 'spots' containing cDNA (complementary DNA)

→ mRNA samples preferentially bind to ('hybridise' with) corresponding cDNA
  - ↳ 'reverse transcription'! (DNA ← mRNA)

Also:
- use fluorescent tags to distinguish different samples
- amplify levels of products via PCR/qPCR.

Preprocessing

Preprocessing is crucial

BUT: we won't go into here

Typical considerations:

- artifacts
- absolute vs relative expression levels
- log transformations

etc

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usually work with

log(relative expression)

From now: take measure as 'given' & just use numbers.

→ Vinod knows more about experimental side!

Data & Experiment Types

We consider **two types** of experiment

1. **Perturbation** (or **comparative**)
   - effect of treatment
   - different tissue types etc

   } multiple sample 'types'; same 'time'

   — eg treated vs untreated
   — cancer or not etc.

2. **Time Series**
   - same cell/tissue etc studied over time

   } one sample type, multiple times

Idea:
- time has natural order
  - continuous or categorical ordinal
- treatment/class not necessarily ordered
  - cancer or not etc

BUT essentially

- just different independent vars, same basic ideas
- also, often want to combine, eg compare two time series from different treatments at same times

Perturbation / comparative

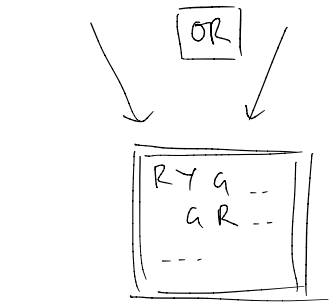
- Have both a treatment & control (& at single time)

Variations:

(A). Red dye — Treatment; Green dye — Control; separate
- different chips

or

(B). OR → Competitive (typical)
- same chip for both treatment & control
- compete to hybridise with probes



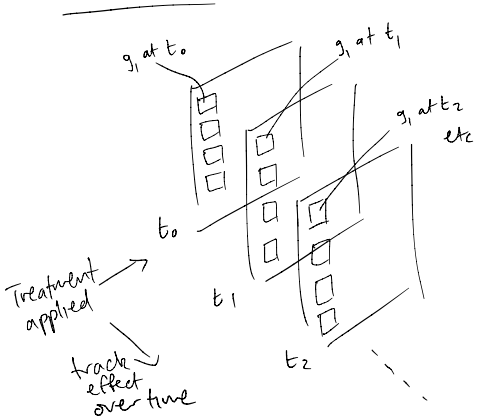
where:

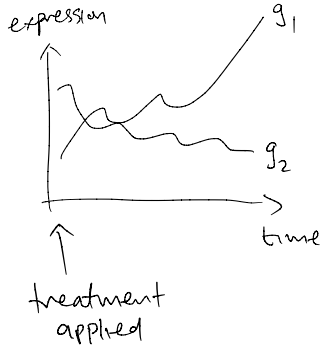
direction of treatment relative to control → Who wins?
- ↑ : red
- ↓ : green
- — : yellow

→ resulting colour on chip.

ie red: expression ↑ for treatment
green: expression ↓ for treatment
(rel. to control).

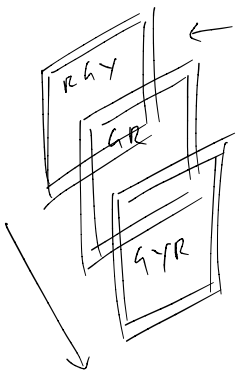
Time Series





- treat one sample type at t_0 (say)
- new chip for each time
- track resulting expression patterns over time.

Combined:



← each array has both treatment & control (or some other comparison)

+

track over time → (new array per time)

Data format : **Gene expression matrices**

→ A general format that we can put both time series & perturbation/comparative into:

	Exper.1	Exper.2	Exper.3	...
Gene1				
Gene2				
Gene3				
⋮				

Eg experiment 1 : time t_0, control

experiment 2 : time t_1, control

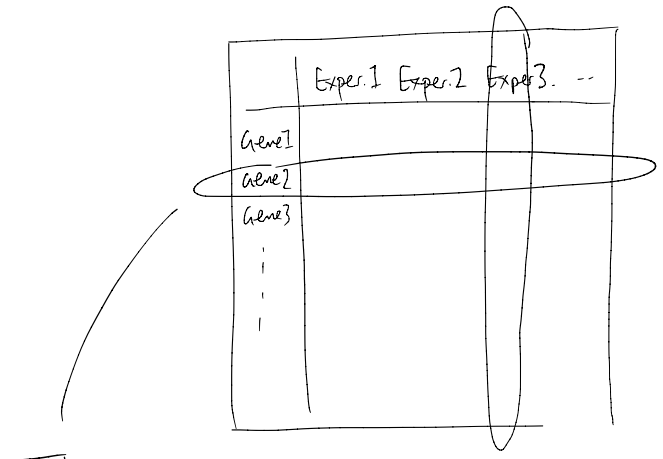
⋮

experiment n+1 : time t_0, treatment

experiment n+2 : time t_1, treatment etc.

(Warning : many 'data array' formats in stat./ eg R, Python etc are the transpose of above : genes are col, exp. are row)

Gene expression matrices



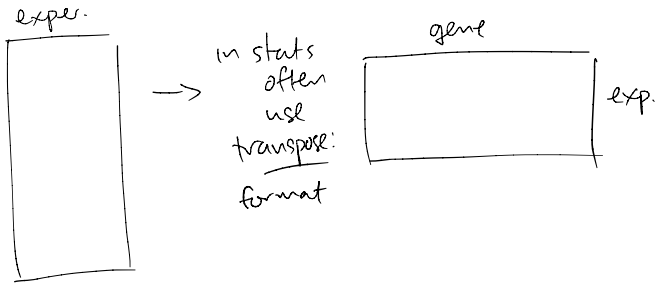
Column: a particular experiment, ie an array (gene chip/microarray) of all genes.

row: a gene expression profile over experiments for a given gene.

Typically : ⁂ genes >> experiments

unknowns data

Here:



Questions / Problems

Q: which genes 'cause' difference between control & treatment / two samples for comparison?

→ many more genes than experiments

→ overfitting issues if try to explain via single genes!

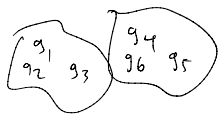
eg one difference (cancer or not) → 1000 possible explanations!

→ problem for naive/traditional stat. inference!

└ motivation for many modern stat/ML methods...

One way to tackle:

- focus on sets/clusters of genes

eg 

→ smaller number of these groups

→ also relates to idea that genes work together & co-express

Typical analyses

1. Clustering: similarity analysis (eg over time)

2. Perturbation: linear control analysis based (SS).

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1. **Clustering**

- a form of **unsupervised learning** for pattern discovery

- need a notion of 'distance' however
  └ user input (see eg prev. lectures & below)

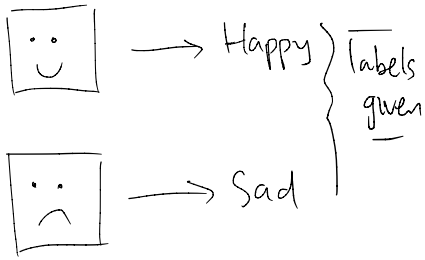
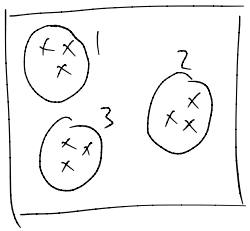
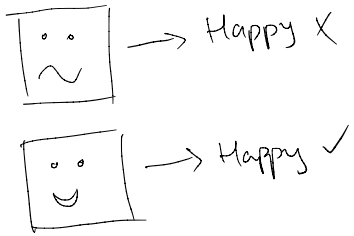
For fun (not examinable):

Defining idea of 'distance' & related:

distance	metric	dissimilarity
i) $d(x,y) \geq 0$	i–iii) &	i–ii) &
ii) $d(x,y) = d(y,x)$	iv) $d(x,y) = 0$ iff $x = y$	iii)*: $d(x,y)$ increases monotonically as x & y more 'dissimilar' (subjective)
iii) $d(x,x) = 0$	v) $d(x,y) + d(y,z) \geq d(x,z)$	

(Terminology)

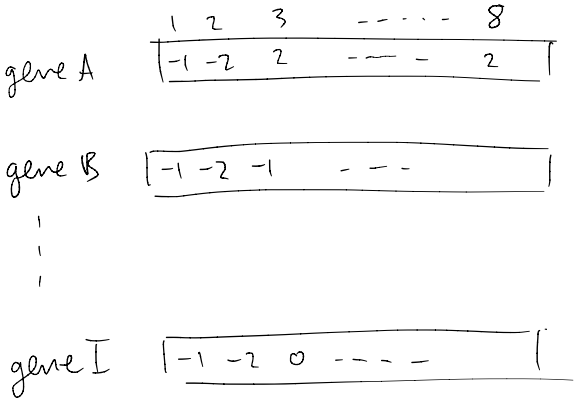
Unsupervised? Note on 'learning types'

Supervised	Unsupervised
$X \longrightarrow Y$	X ↻
learns function $X \to Y$	pattern discovery 'in X'
Train: supervisor gives examples	Eg Find clusters
 labels given	
Test: predict on new (unseen) set	→ group 'similar'
	→ no labels given → do need a distance/ similarity measure → in general harder to evaluate (some methods exist ---)

Example: Clustering expression profiles

→ across time &/or experiments (ie profiles of genes: )

Expression matrix:



Q: which genes have 'similar' profiles?

→ define distance ... eg Euclidean (since easy)

Distance between two profiles A, B :

$$d(A,B) = \sqrt{\sum_{i=1}^{n} (y_i^{(A)} - y_i^{(B)})^2} \qquad \text{(Euclidean)}$$

(square root of sum of squared differences)

where
- n : number of experiments
- $y_i^{(A)}$: expression level of gene A in experiment i

Example

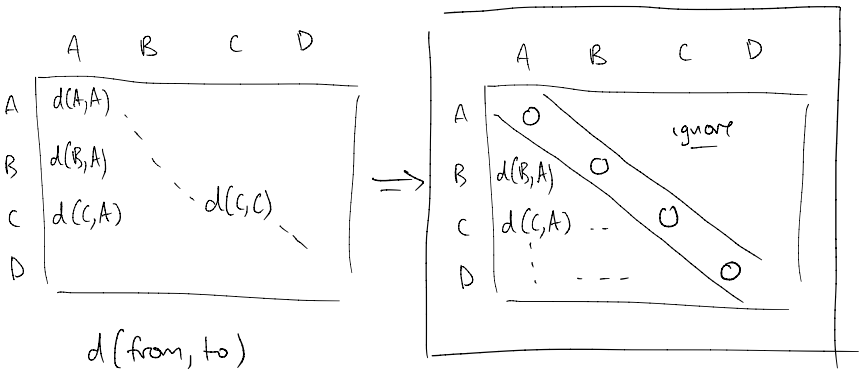
A	1	-1	2	0	} two gene profiles.
B	1	-1	-1	2	

Squared diffs :	0^2	0^2	3^2	2^2	Sum squares!
ie	0	0	9	4	13

so $\boxed{d(A,B) = \sqrt{13}}$

Distance matrices

- we can summarise all pairwise differences between profiles in a **distance matrix** (note : this isn't an expression matrix)



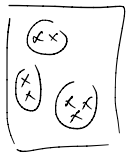
d(from, to)
= d(row, col)

Note : - symmetric, so don't need explicit upper part
ie $d(B,A) = d(A,B)$

- diagonals are zero.
$d(A,A) = 0$ etc.

Distance-based clustering

Goal: group together if 'close' eg:



etc.

Two popular algorithms

- K-means
- Hierarchical

Here: will consider hierarchical

Pseudocode (Hierarchical)

Begin with n observations

Find all pairwise distances

For $i = n, n-1, \ldots 2$:

Find smallest distance

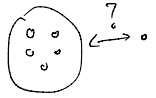
'Fuse' or 'group' together (so n obs → n-1 obs)

Recompute distances to 'fused' cluster *
(see below)

* Distance to cluster?

- multiple types
  - min
  - max
  - average
  - etc...

} 'linkage type'

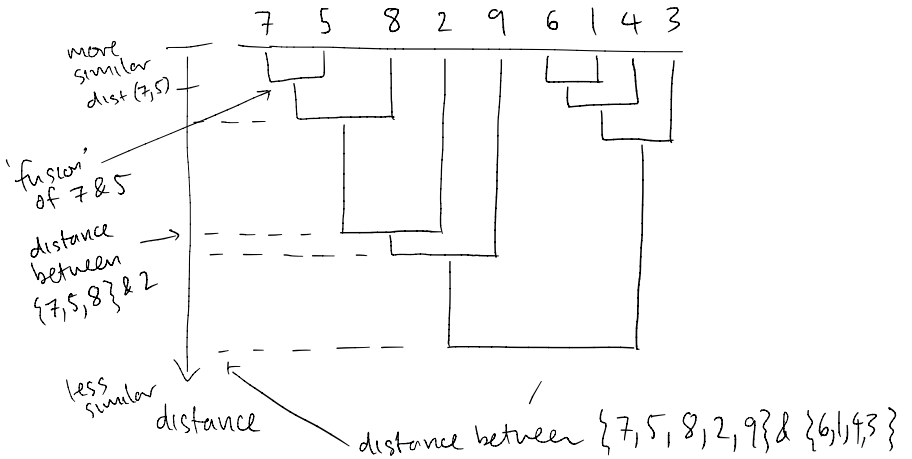


Dendrograms

- summarise results of hierarchical clustering

- Indicate distance between various clusters in 'tree-style' diagram

Example:



- height (y axis) of 'fusion' indicates distance
- for any two clusters, can find where they first fused

Other analysis type.

2. 'Control' analysis via individual perturbations

Consider gene expression matrix again:

	Experiment 1 2 3 ---
gene 1	
gene 2	
gene 3	

Goal: how do genes 'affect' other genes?

Can we deduce 'control network'?

Effect of perturbations

→ Make each experiment a perturbation of a single gene

- Increase transcription rates of each gene in turn
- measure the change in steady state concentrations (expressions of mRNA) for all genes (incl. self).

→ get (co-) 'control coefficients' (eg +1.5 or −0.5)

→ summarise in a regulatory strength matrix

see: de la Fuente (2002) 'Linking the genes' for details.

Effect of perturbations of gene transcription rates on ss. concentrations/expression levels

Result:

- Can summarise in **regulatory strength matrix** R_d
- can use to draw potential gene regulatory networks

R_d =

	Experiment: rate ↑ of.. gene 1	gene 2	...
Δgene 1 level	−1	0.5	— —
Δgene 2 level	−0.2	1	
⋮	⋮	⋮	⋱

↑ normalised change in expression level

Example

$$R_d = \begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccc} A & B & C \end{array} \\ \begin{bmatrix} -0.5 & 0 & 0 \\ 0.1 & 0 & 1.2 \\ 0.8 & -0.1 & 1 \end{bmatrix} \end{array}$$

rate increase

s.s. conc. change

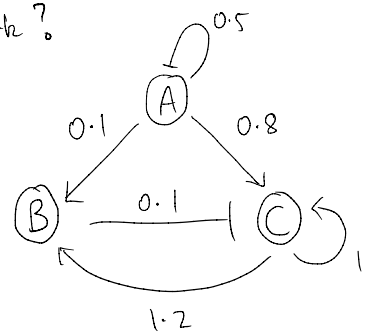
Interp:

effect of..

on [ ]

Q: Network?

A:



⊣ negative
← positive

negative auto (self) regulation: A

positive auto regulation: C