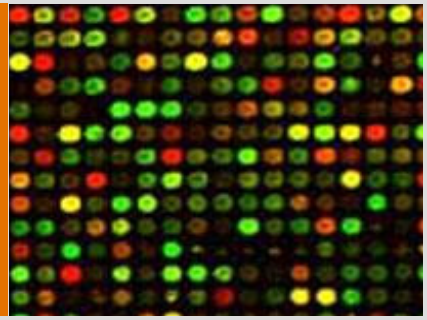

Data Mining Techniques for DNA Microarray Data

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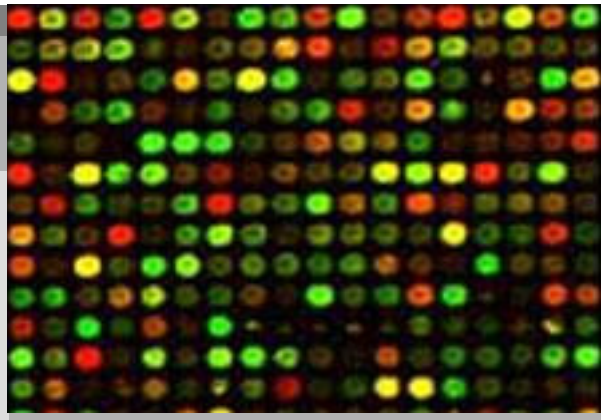
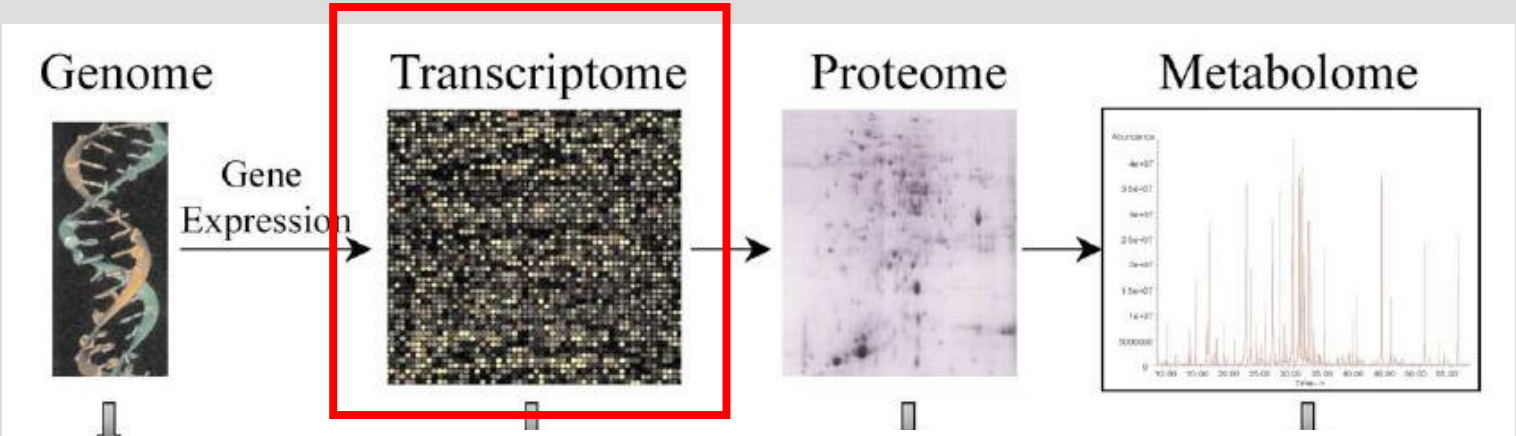
PRESENTATION STRUCTURE





DNA sequence databases

Transcription analysis is the most mature high-throughput analytical tool, but we still don't understand the full impact of the information.

How can we analyse the transcriptome?

Identification of Gene Function
Gene Annotation
Identification of Pathways

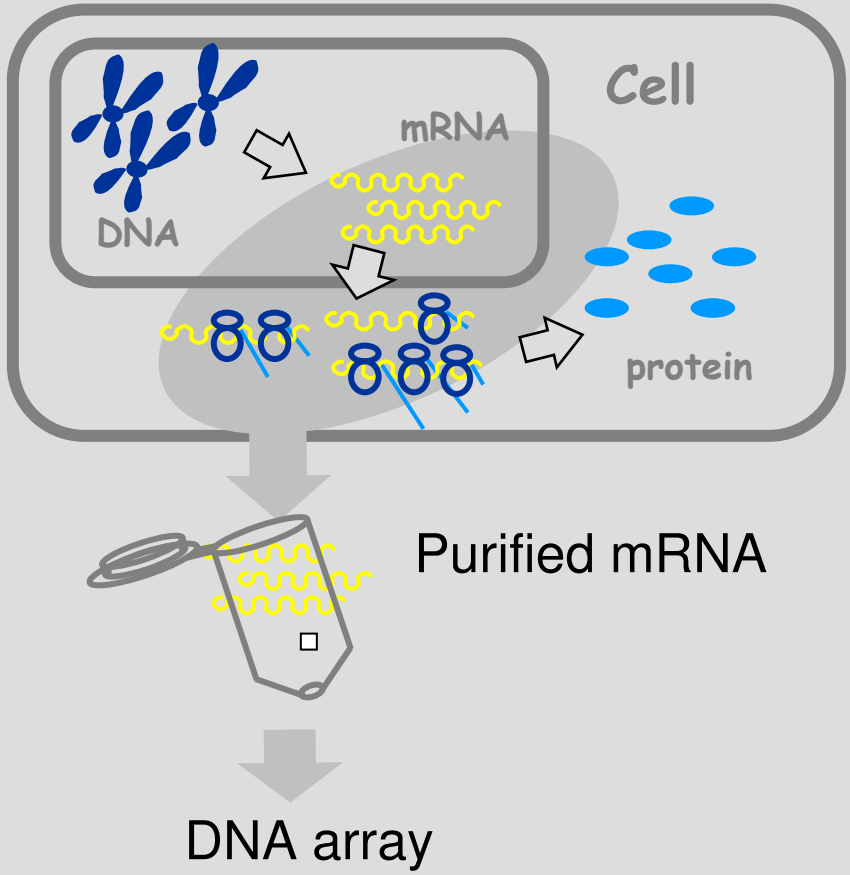
Biological Knowledge, Plant Improvement, Genetic and Metabolic Engineering

The *Saccharomyces cerevisiae* genome has ~6,000 genes …

The human genome has more than 20,000 genes …

What are the functions of these genes?

How can we measure the transcription level of <u>all</u> genes and <u>compare</u> them?



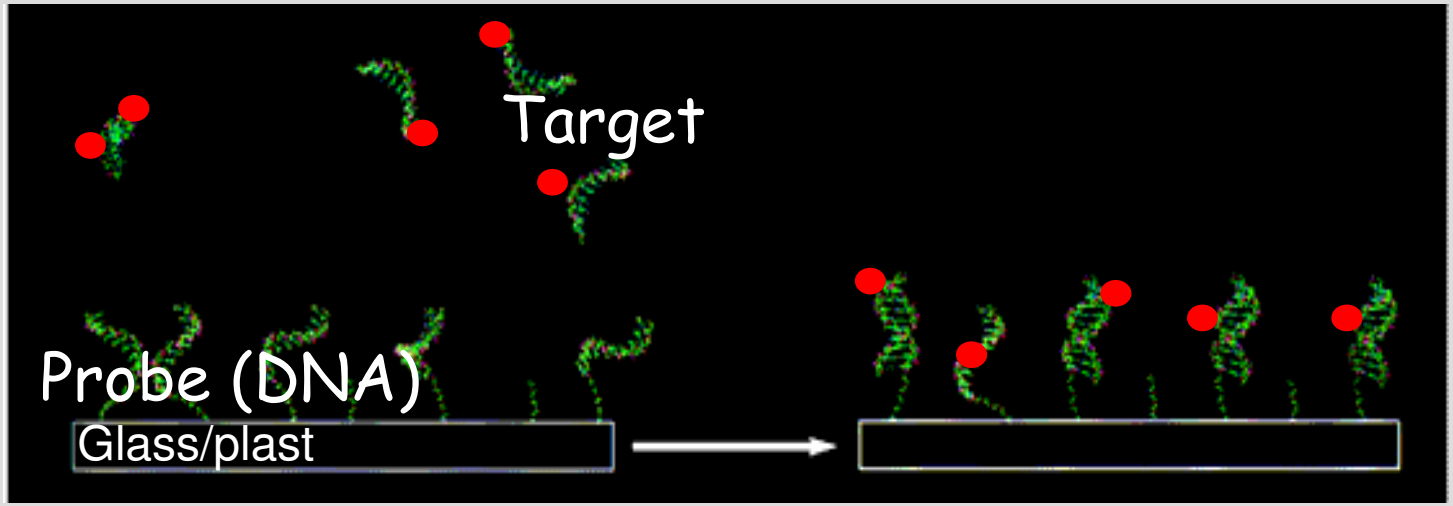
- Identification of complex genetic diseases
- Drug discovery and toxicology studies
- Mutation detection
- Pathogen analysis
- Differing expression of genes over time, between tissues, and disease states

Two different Technologies:

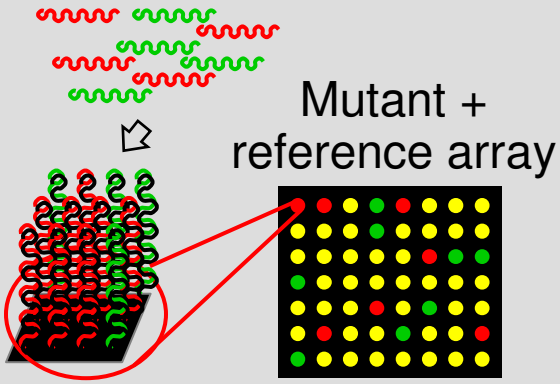
Off-chip - pre-synthesized oligonucleotides, with usually > 100 nucleotides, are spotted or printed on the surface of the support (usually polylisine coated glass slides). Can be made in-house. Also known as cDNA arrays.

On-chip – smaller oligonucleotides (20-80-mer) are synthesized in situ using several methods like barrier methods, ink-jet or photolitographic appraches (example – Affymetrix GeneChips)

These 2 different approaches also imply different experimental design strategies.

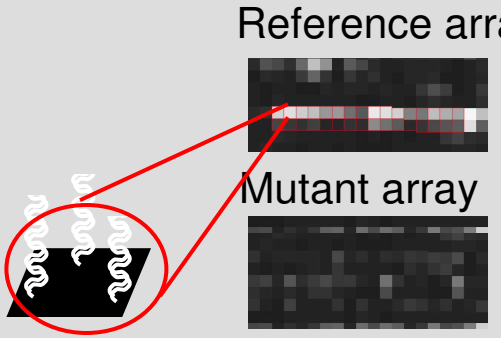


cDNA array



Cy3 = cyanine 3 = green
Cy5 = cyanine 5 = red

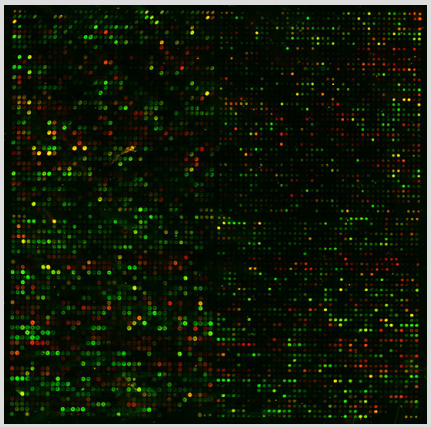
Oligonucleotide array

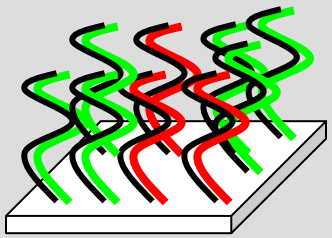


Technology

cDNA arrays

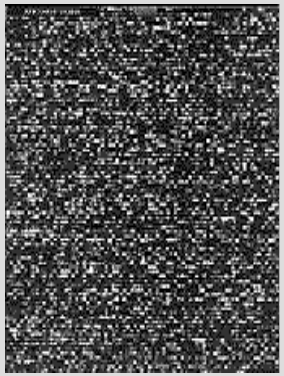
Long probes generated by PCR

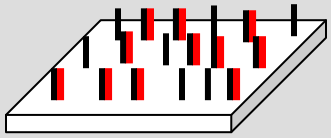


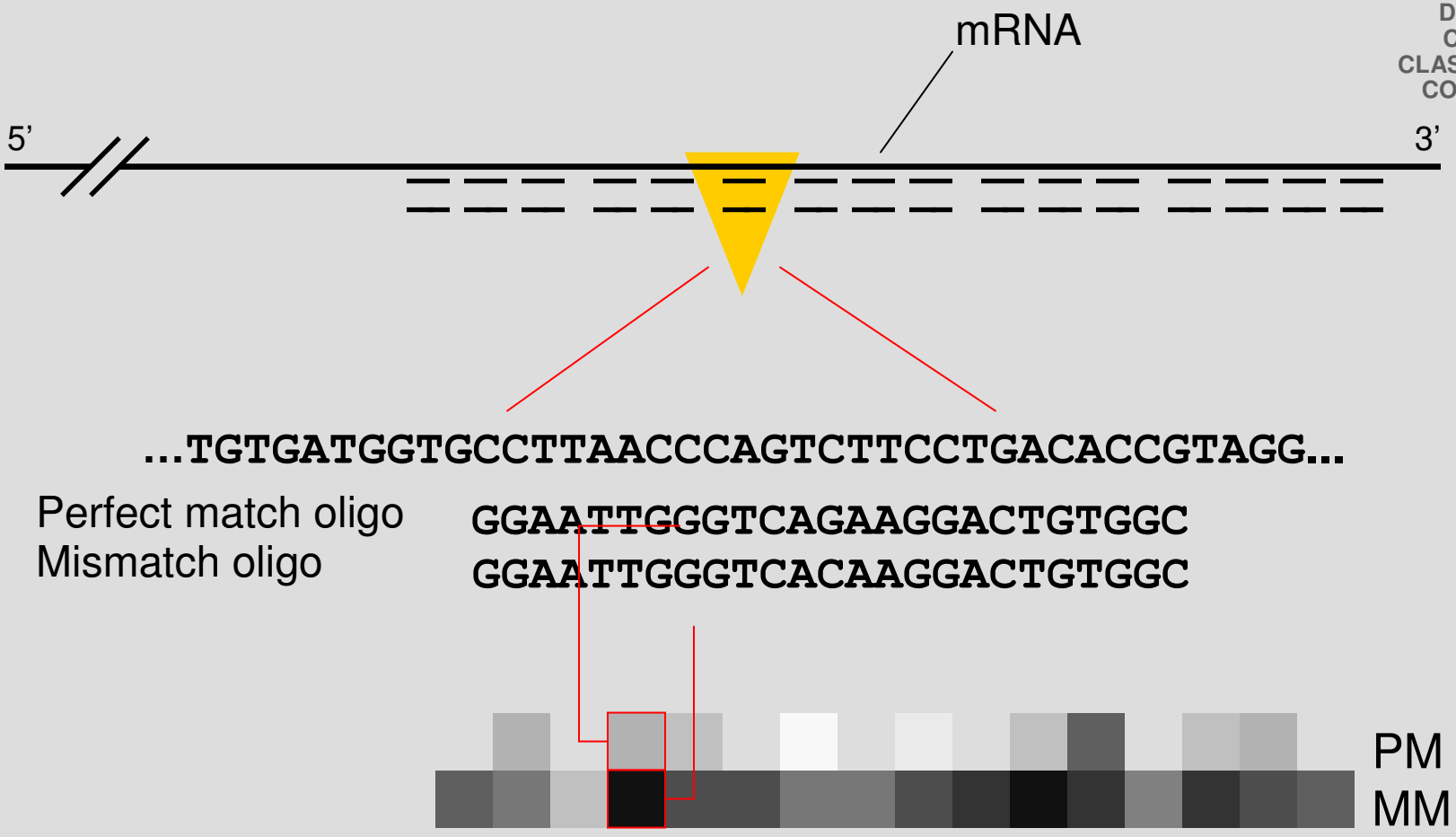


oligonucleotide arrays

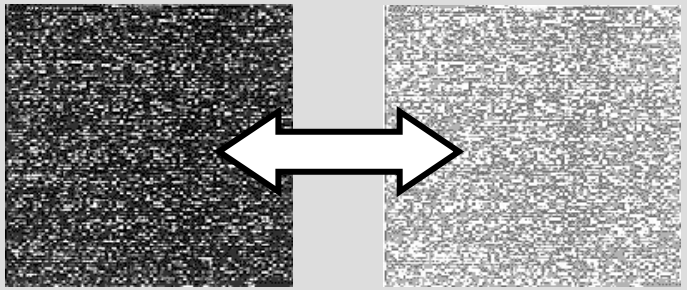
Short probes synthesised on the support







Expression level = average difference = (PM-MM) / (#probe pairs in Avg.)



Missing values treatment:

Do nothing

Substitute missing values with mean / mode / median / specified value

Use imputation by k-nearest neighbours

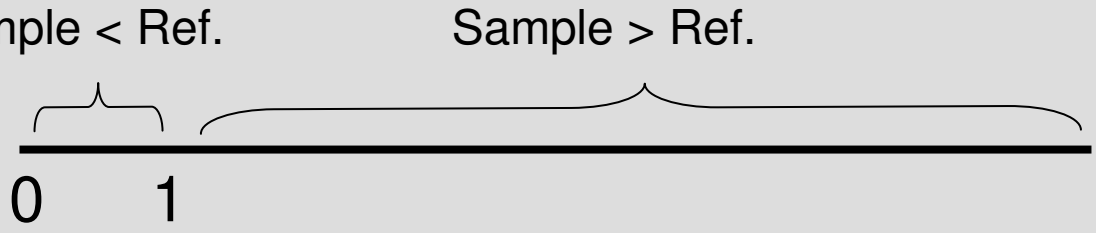
Background correction:

expression level = spot intensity – background intensity

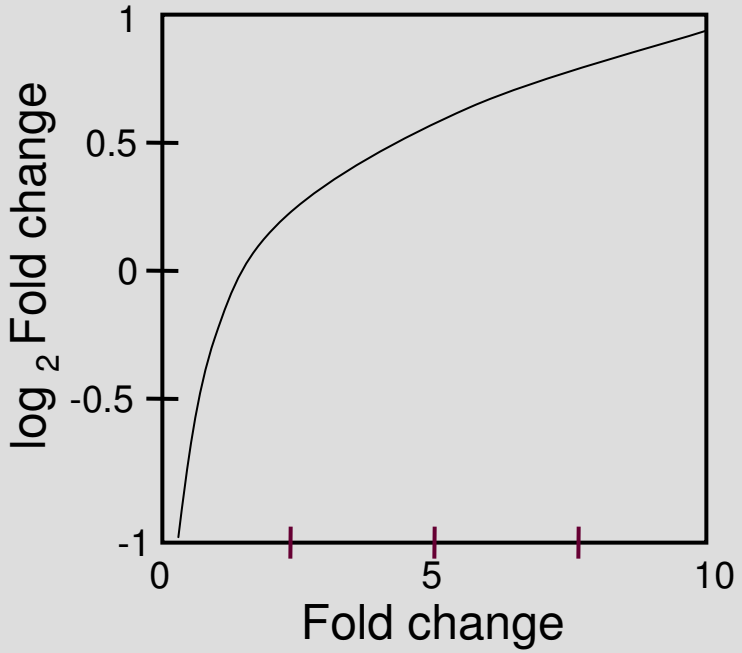
Potential problems: spot intensities lower or equal than the local background

Fold change = Expression level experiment / Expression level control

Problem:



A common approach is to log-transform data



Why to remove flat patterns:

We cannot distinguish signal from noise in such genes

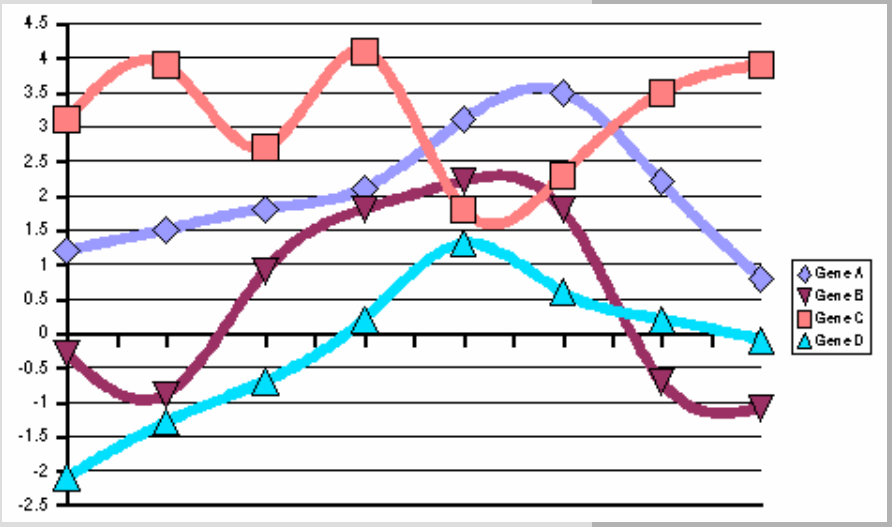
Flat patterns correlate well with nearly anything

What is "flat"?

- Definition based on the number of peaks
- Based on a measure of variation (e.g. standard deviation)

Option:

Only filter genes with values near 0 in all experiments - use root mean square

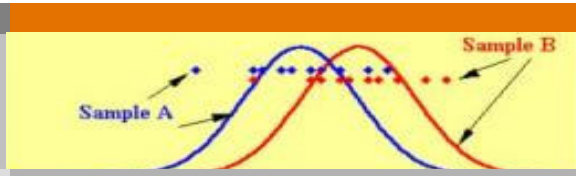


It is common to re-scale the values in order to achieve values with an average of 0 and a std deviation of 1

This normalization will help in the task of clustering !!

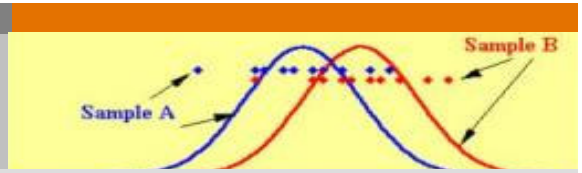
How ?

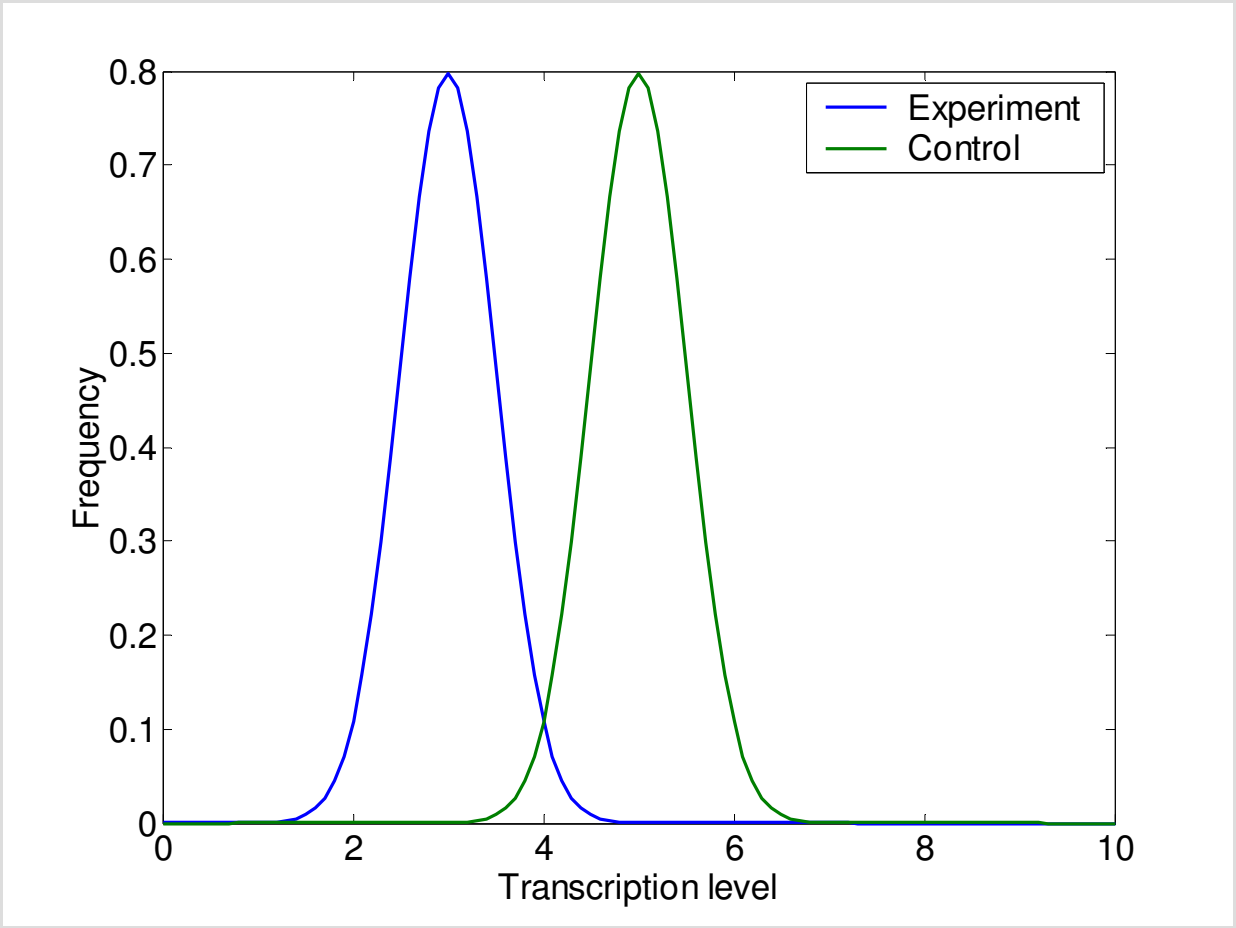
Subtracting the average value of the gene expression to each individual value and dividing the result by the standard deviation



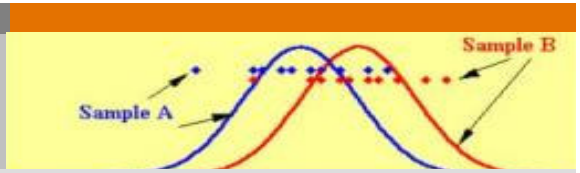
In terms of biological research it is very important to **identify the set of genes that have different levels of expression** comparing two experimental conditions (e.g. healthy cell vs cancer cell, wild type vs mutant).

The identification of genes that are differentially expressed relies on Statistical **tests of hypothesis**, based on the assumption that the values of gene expression are “generated” following a normal distribution.





Hypothesis: Is the average transcription level of Experiment and Control identical?



An example with low and high P-values

Gene	Control 1	Control 2	Control 3	Exp 1	Exp 2	Exp 3	P-value
1	8.41	8.45	8.37	9.29	9.39	9.20	0.003
2	8.82	8.93	9.20	9.84	9.79	9.56	0.004
3	11.70	11.70	11.66	12.03	11.99	12.05	0.005
...	...	...	...	...	...	...	...
N-2	6.05	6.05	6.05	6.05	6.30	5.73	0.423
N-1	8.32	8.26	8.23	8.77	8.08	7.73	0.733
N	10.93	11.14	11.10	10.71	11.59	10.99	0.827

What happens when we are testing many times?

P is the probability of making an error when testing once…

When testing *R* times the probability of making an error increases to

$$P_{error}(R) = 1 - (1 - P)^R \approx RP$$

Bonferroni suggested $P_{corr} = P/R$ as a better cutoff-value to compensate for multiple-testing

A number of Statistical techniques can be applied for more elaborate analysis:

- One way analysis of variance (**ANOVA**)

Analyse the mean and variance of gene expression across groups of conditions

- Two factor ANOVA (**TFA**)

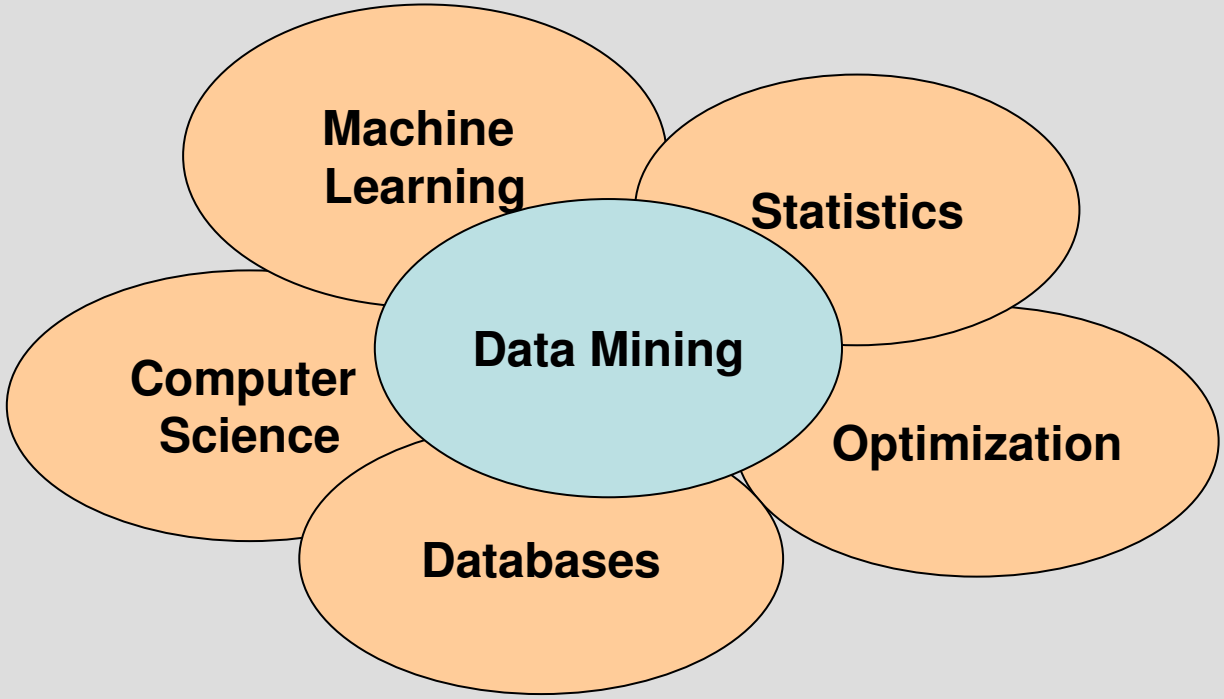
Used to find genes whose expression is significantly different over two factors

- Significance analysis of microarrays

Used to pick out significant genes based on differential expression between sets of samples (one class, two class, multiclass)

What is Data Mining ?

The analysis of datasets, typically of **huge dimensions**, aiming to discover previously and potentially interesting **unknown relationships** in a way understandable to the user.



Tasks of Data Mining:

Exploring the data – typically visualization tools

Clustering – **Unsupervised learning**

The aim is to group the data into clusters, finding sets of related itens

Association rule induction

The aim is to find IF ..THEN ... Rules that apply to a significant subset of records

Prediction – **Supervised learning**

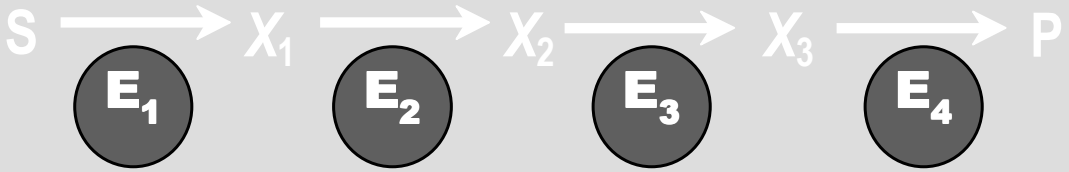
Classification, regression, time series analysis

All these tasks have applications in Microarray Data Analysis !!

Motivation: co-regulated genes show a "similar" behaviour

"Guilt by association" → if unknown gene *i* is similar in expression to known gene *j*, maybe they are involved in the same/related pathway

Metabolic pathway



Aim

To find sets of genes that are co-expressed over a set of conditions (experiments) – **UNSUPERVISED LEARNING**

Assumption (biological)

Similarity in gene expression implies some form of regulatory or functional similarity

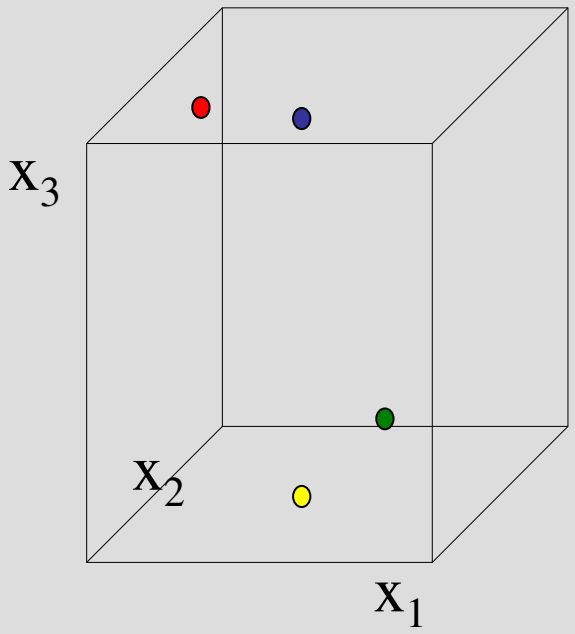
An “expression vector“ is defined for each gene

The expression values are the dimensions (x_1,x_2, ..., x_n) in “expression“ space.

Each gene is represented as one n-dimensional point

Two genes with similar behaviour are “spatially“ nearby

Similarity is inversely proportional to spatial distance



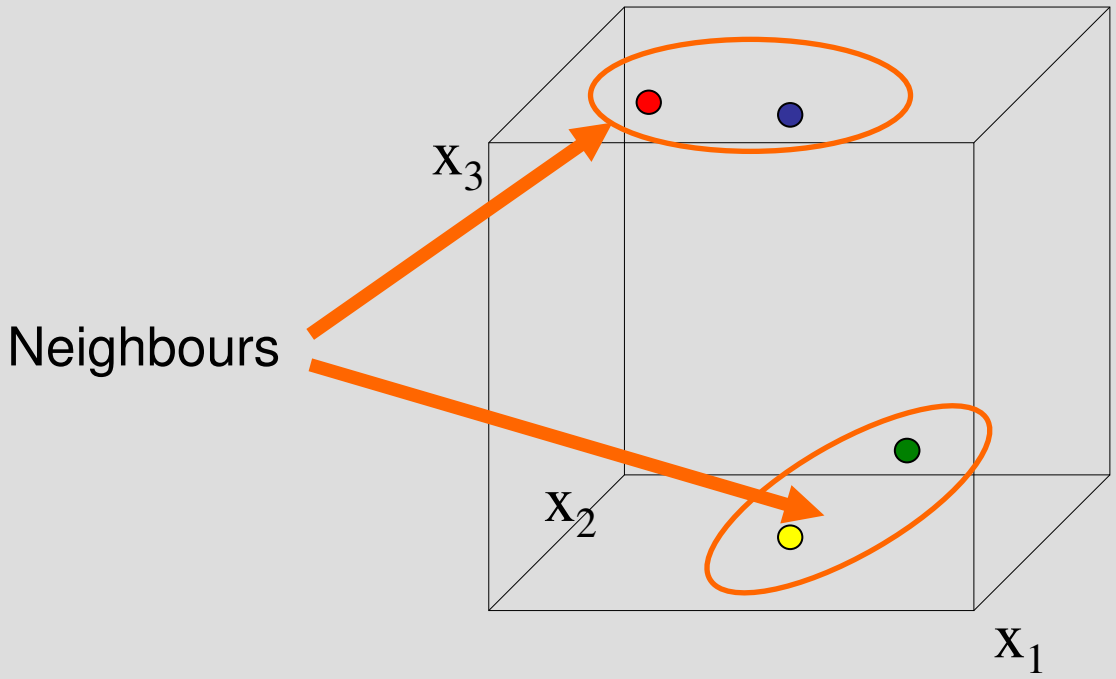
But, what is similarity?

There exists no *a priori* definition of similarity

Different similarity measures can be used

Different patterns are “observed“ with different similarity measures

How similar are the red and the blue spheres?



Manhattan distance

$$d(i,j) = |x_{i1} - x_{j1}| + |x_{i2} - x_{j2}| + \ldots + |x_{ip} - x_{jp}|$$

Euclidean distance

$$d(i,j) = \sqrt{(|x_{i1} - x_{j1}|^2 + |x_{i2} - x_{j2}|^2 + \ldots + |x_{ip} - x_{jp}|^2)}$$

Pearson‘s correlation coefficient

It is the dot product of two normalized vectors, or the cosine between two vectors. It measures the similarity in the shapes of two profiles, while not taking the magnitude of the profiles into account and therefore suits well the biological intuition of coexpression.

Comparing distance

Comparing shape

Optimization problem

To find a set of clusters that accomplish the following aims:

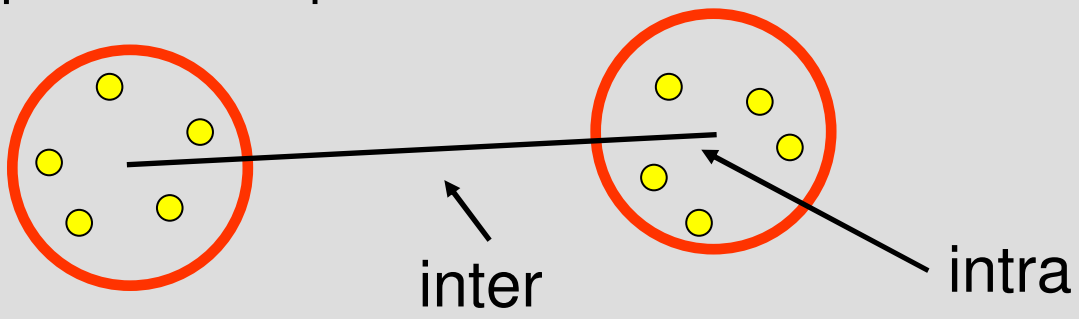
- **Homogeneity**

Elements within a cluster should be “near”, i.e., should have small intra-cluster distances

- **Separation**

Elements of distinct clusters should be “far”, i.e., should have large inter-cluster distances

Since these aims are sometimes contradictory the problem is quite difficult to handle !!



Clustering experiments

The aim of clustering can also be to **cluster experiments**, i.e., to find sets of experiments where the expression of the genes is similar

In this case, we have for each experiment a vector with the values of expressions for the whole set of genes

The algorithms are the same altough the dimensions are different imposing distinct problems to the algorithms

Classes of algorithms

- Hierarchical clustering
- K-means
- Self-organizing maps and Self-organizing trees
- Evolutionary/ memetic algorithms
- Quality-based algorithms
- Biclustering
- ...

- Almost a *de facto* standard, enjoys an enormous popularity

- The same concepts are applied in the construction of phylogenetic trees

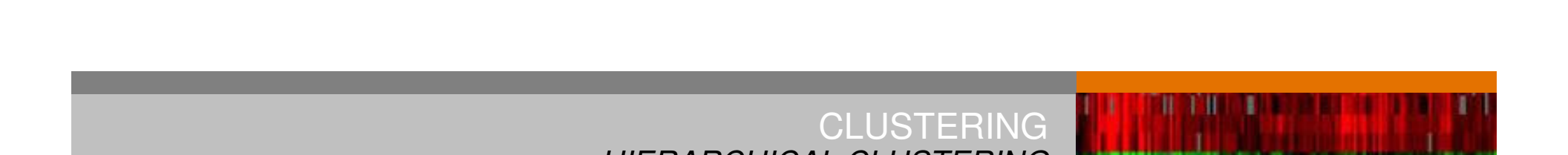
The basic idea – agglomerative HC

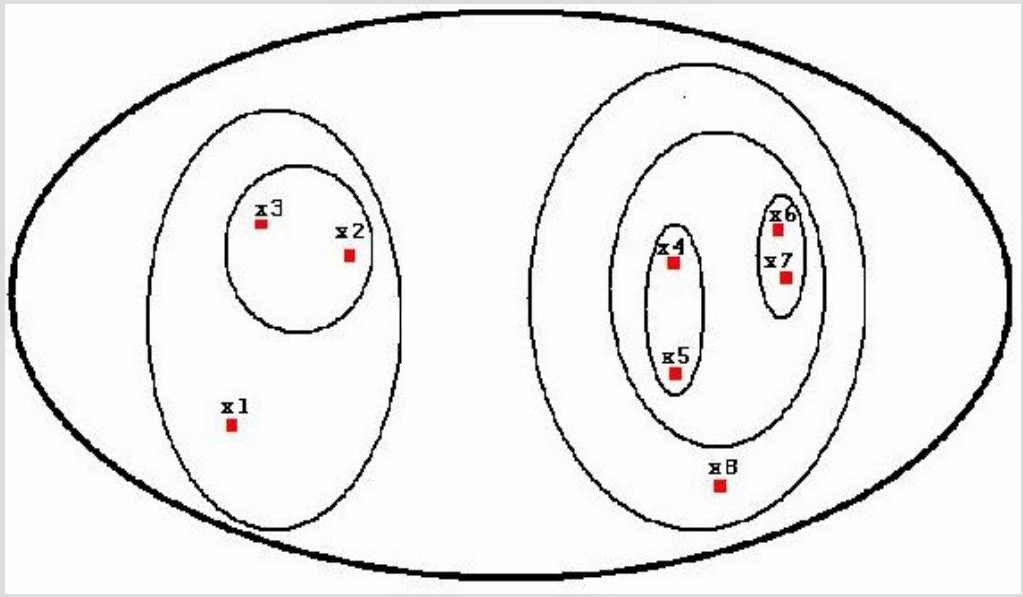
Start with a cluster for each gene

In each step, the pair of clusters with the minimum difference is merged

The process proceeds until a single cluster is obtained

The final clusters are obtained by cutting the tree at a certain level





Venn diagram

One alternative is **divisive hierachical clustering**:

- Start with a single cluster

- Divide one cluster and create two new clusters until every cluster has a single gene.

Distance measure between two clusters

- **Single linkage (nearest neighbour)**

Given by the distance between the two closest points

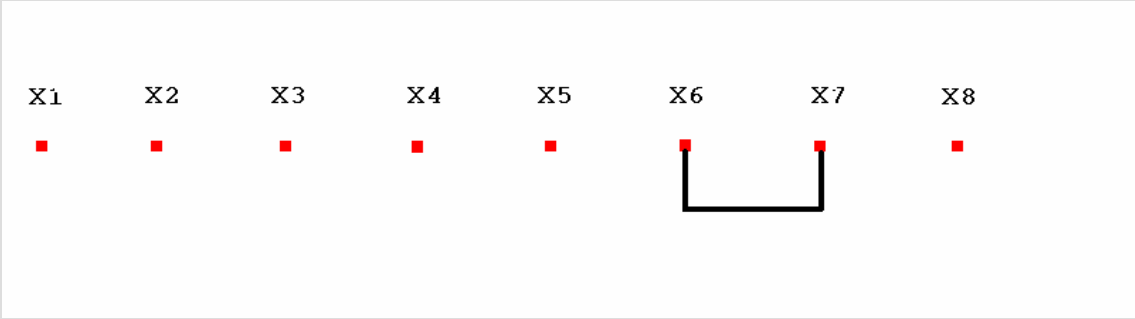
- **Complete linkage**

Given by the distance between the two furthest points

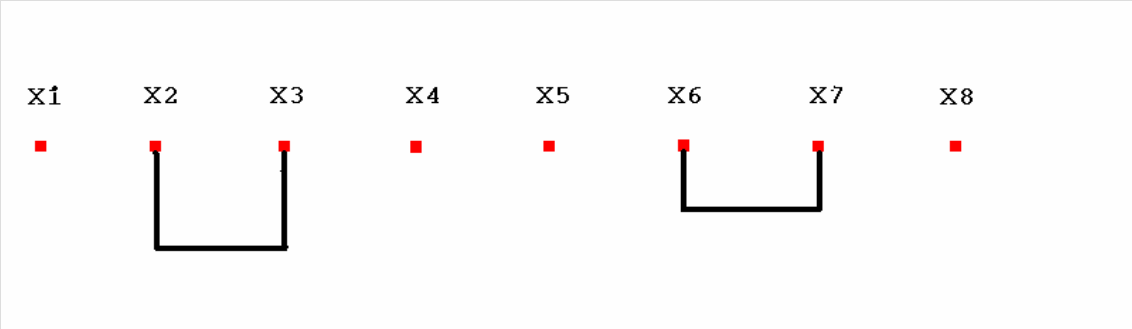
- **Average linkage**

Average distance between the points in the cluster

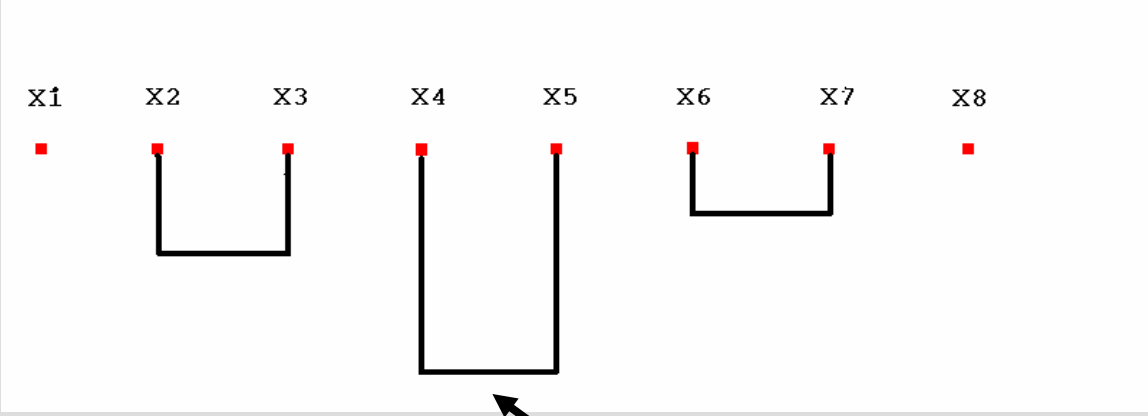
Nearest neighbour, level 2, k = 7 clusters



Nearest neighbour, level 3, k = 6 clusters

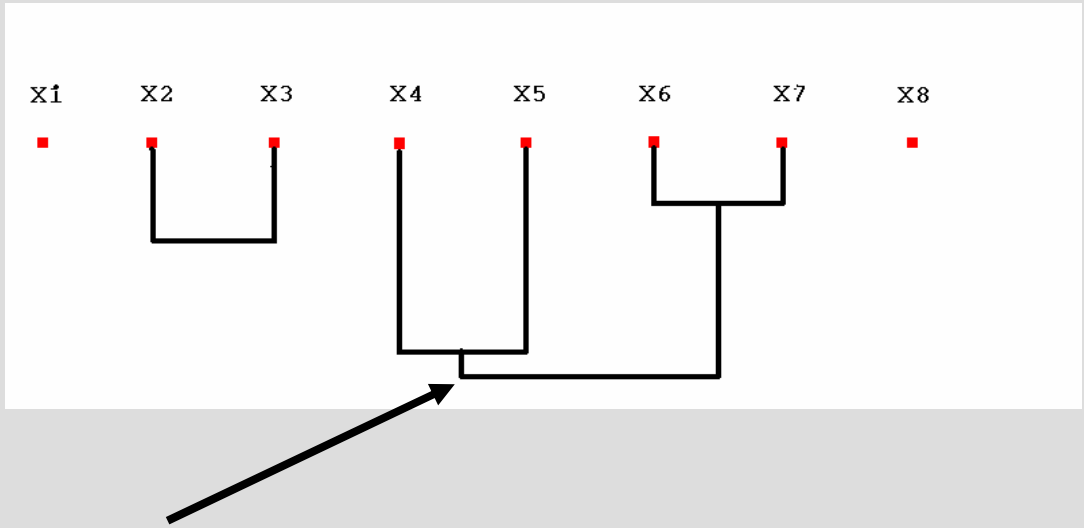


Nearest neighbour, level 4, k = 5 clusters



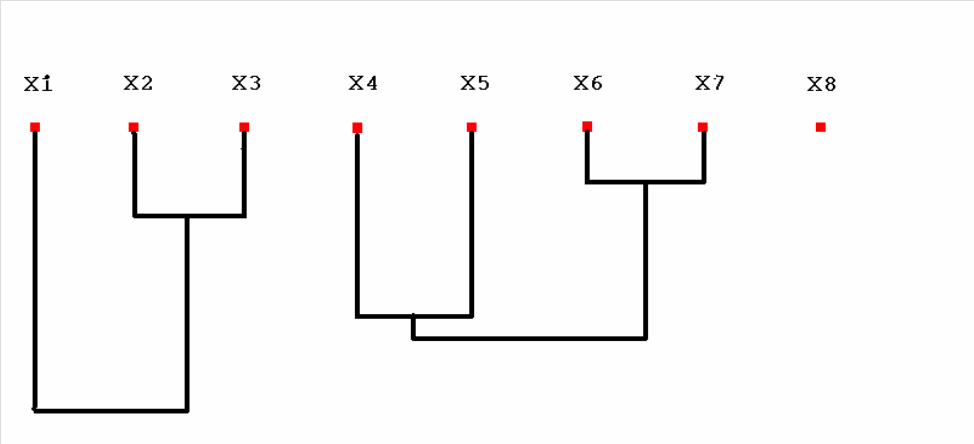
The height of the “U” indicates the distances between two clusters

Nearest neighbour, level 5, k = 4 clusters

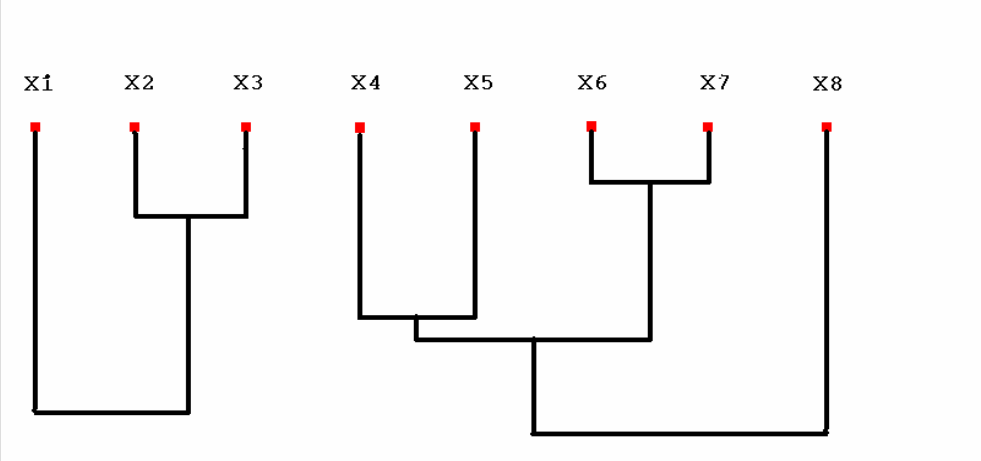


The centre of mass distance of X_4 and X_5 is close to the centre of mass distance for the new cluster with 4 members.

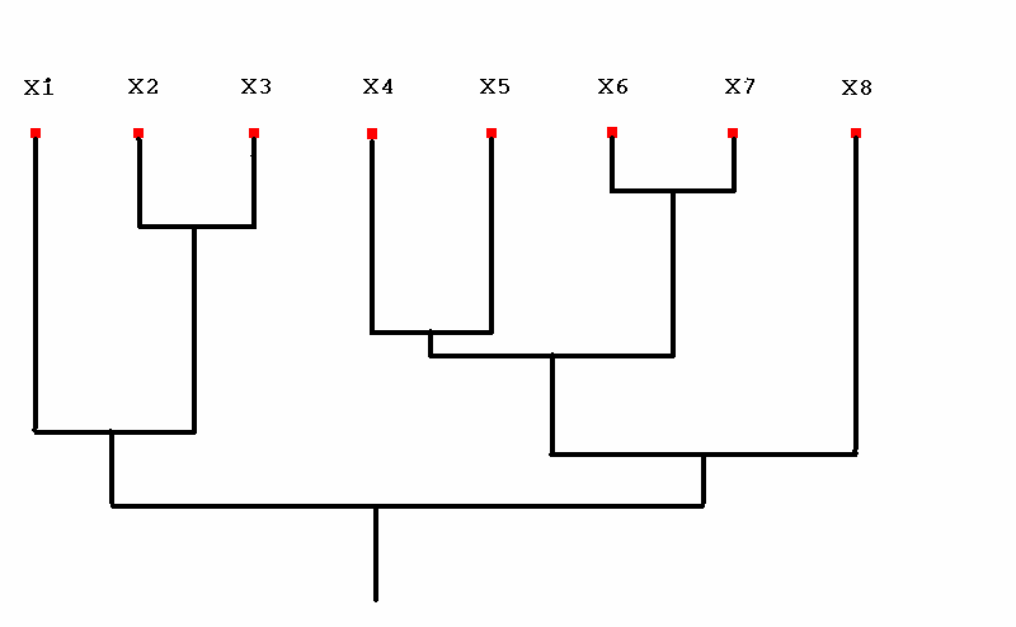
Nearest neighbour, level 6, k = 3 clusters



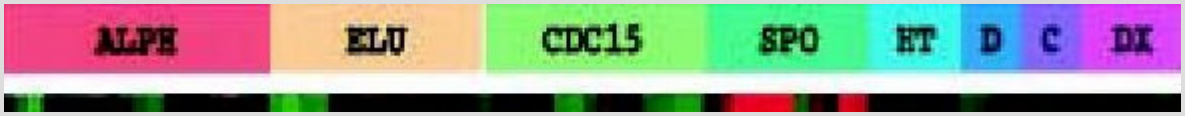
Nearest neighbour, level 7, k = 2 clusters

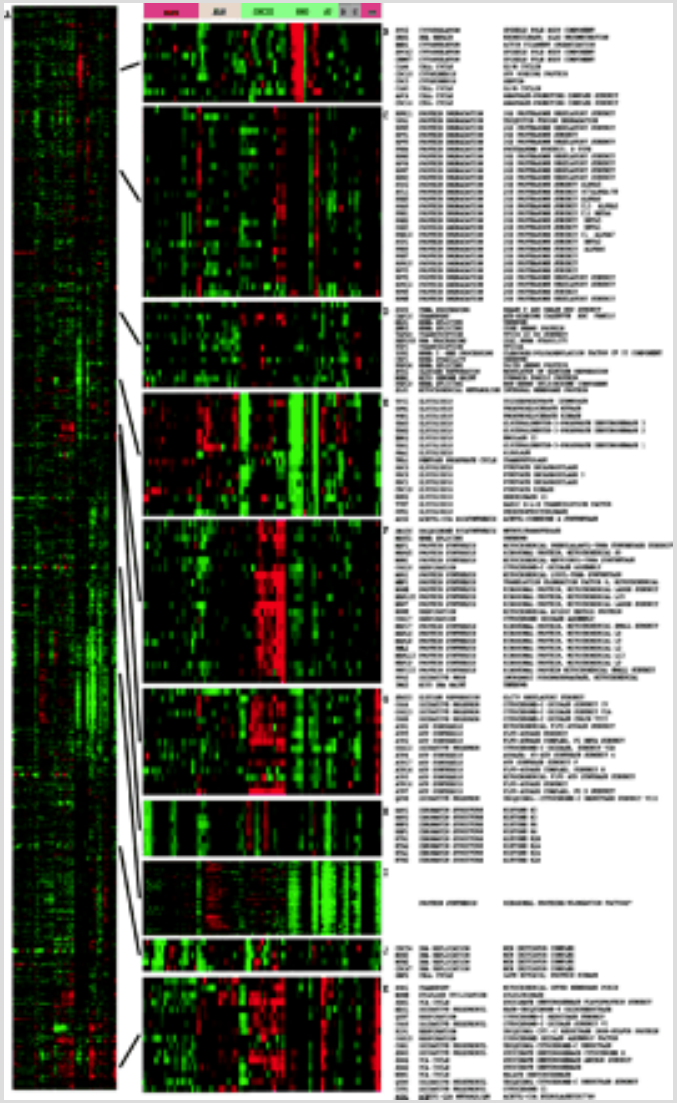


Nearest neighbour, level 8, k = 1 cluster



CLUSTERING
HIERARCHICAL CLUSTERING





Spindle pole body assembly

The proteasome

mRNA Splicing

Glycolysis

The mitochondrial ribosome

ATP synthesis

Chromatin structure

The ribosome and translation

DNA replication

TCA cycle & respiration

An example:
8 experiments

10 clusters

K-means doesn't stand for an algorithm but it is a formulation of the problem:

The microarray clustering task is formulated as a ***minimum sum-of-squares clustering problem***, where the purpose is to minimize the average of the square of the distance between each point an the centroid of the cluster it is assigned to.

The simplest algorithm to solve this problem is the **Lloyd algorithm**.

Lloyd Algorithm:

K – the number of clusters is given

1. Initially, K centroids for the clusters are randomly generated within a given range
2. The genes are assigned to the cluster with the nearest centroid
3. If there has been no changes in 2 the process is finished. Else the centroids of each cluster are re-calculated as the average of the assigned genes and return to 2.

When the process stabilizes the result can be regarded as a membership function for each cluster.

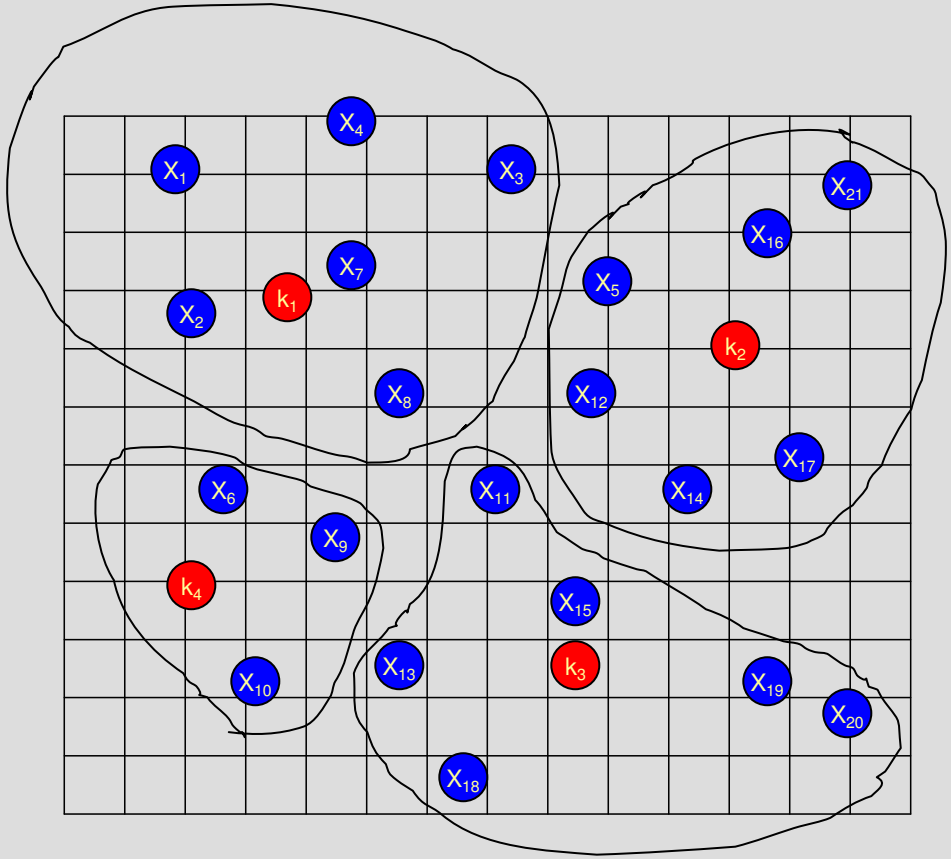
- One of the most popular clustering algorithms
- A partitioning method
- It has been around for a while

  Has many variations

  Has been given many different names (reinvented)
- Simple to understand and implement
- Since it is a **local optimization method**, it is prone to reach local minima. One solution is to run it a number of times and choose the best overall result.

Initialization

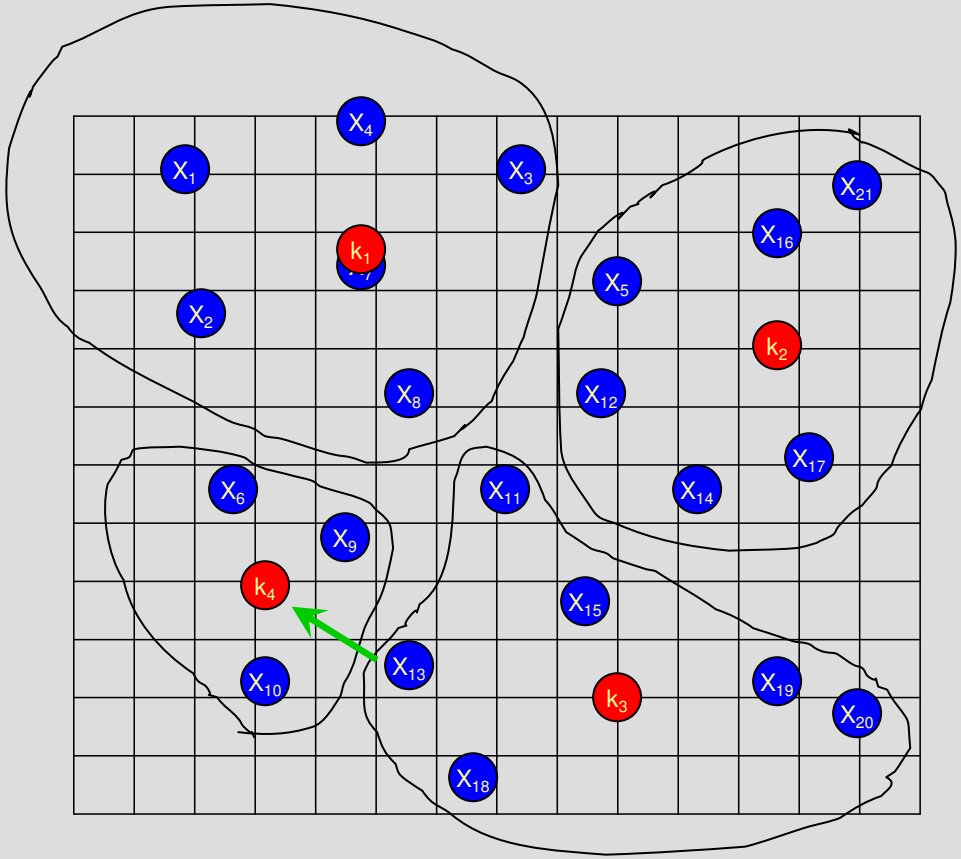
Clustering in 2 dimensions



Initial position of cluster centres is random.

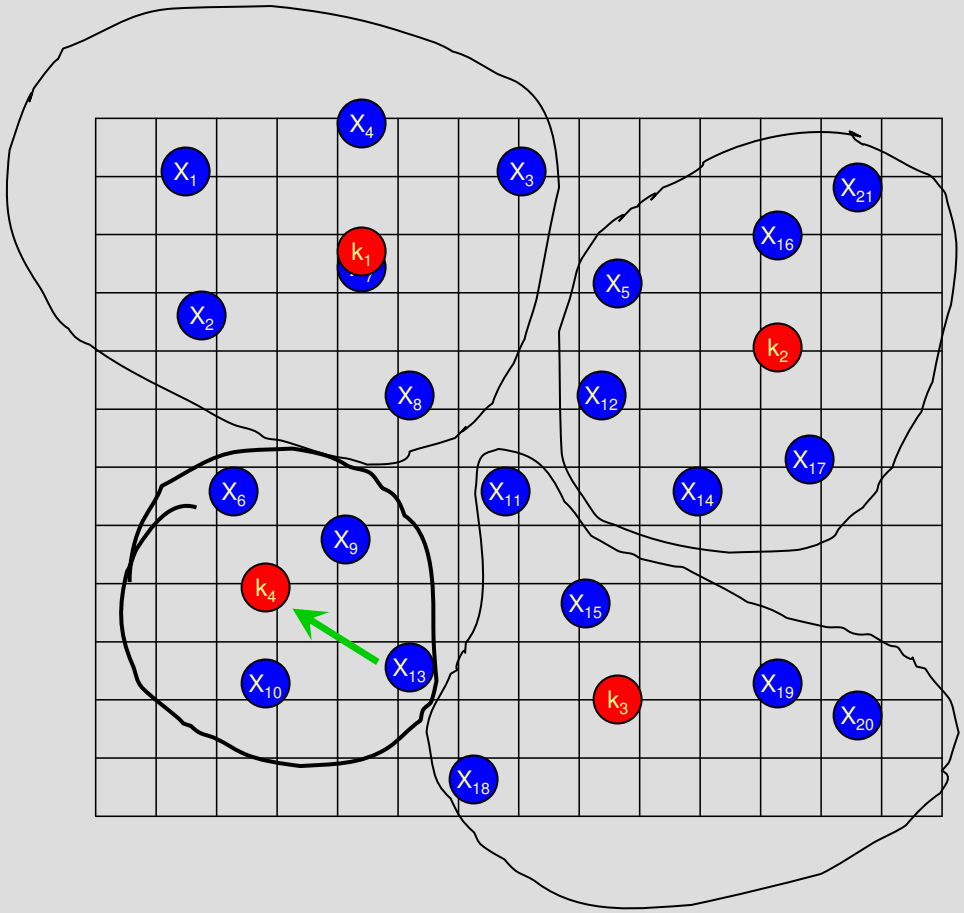
Calculate the *mean* of each cluster

Step 1



Reassign each record to one of the *K* means

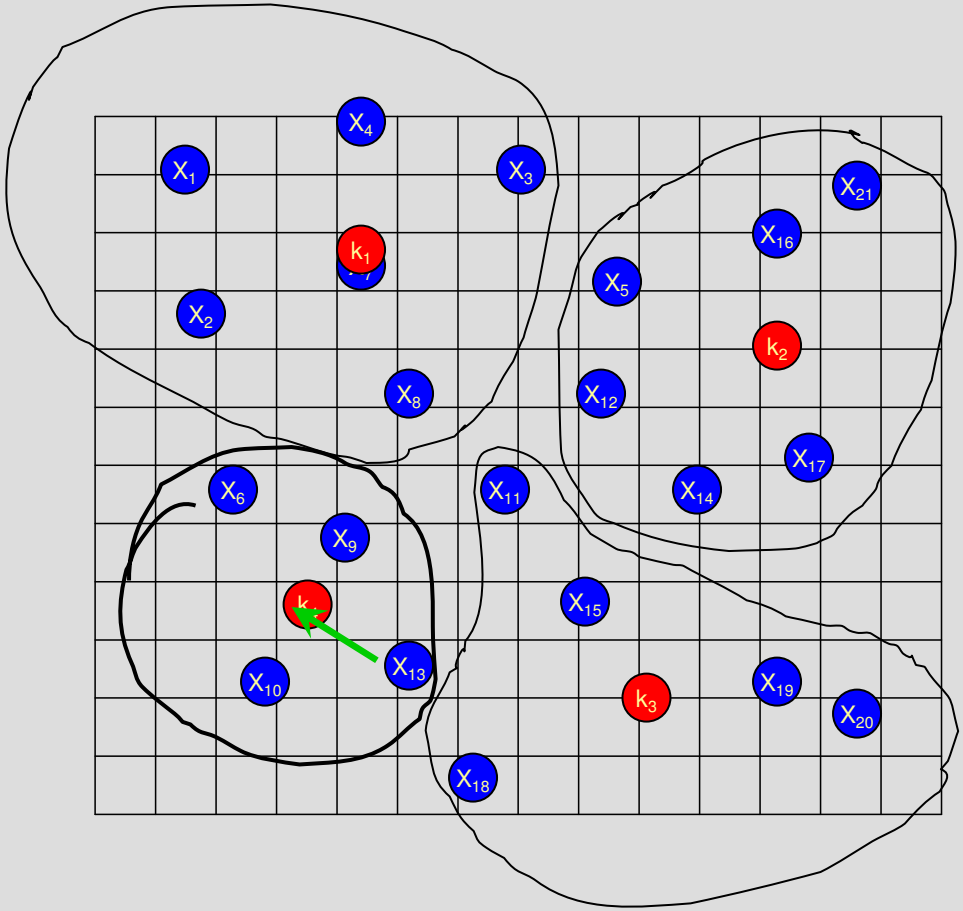
Step 2



Cluster centres are shifted to the centre of data points assigned to this cluster centre.

Step 3

Cluster centres are shifted to the centre of data points assigned to this cluster centre.



Iteration of step 1 to 3 until cluster centres are not shifted anymore. Algorithm terminates!

How to set K?
Very important parameter
- Too large: One cluster per record
- Too small: One cluster for all records

There is a “natural” number of clusters in the data

Many different ways of setting K
- Can try a few and see
- Can start with a few clusters and split the large ones
- Can start with many and merge the small ones

K-means cannot deal with overlapping clusters
- No degree of membership

Problem with outliers
- Can be dealt with in preprocessing

A **Self-organizing map (SOM)** is a model that allows the mapping and visualization of high-dimensional input data (the genes in this case) into an output map of neurons.

The outputs are organized in a two-dimensional grid of neurons.

Each output neuron is represented by a prototype vector.

A process of training involves the changing of the prototype vectors according to:
-They are initially random
- At each step, each input vector is associated with an output node
- The prototypes are updated to get nearer the sum of the inputs it is associated to and away from the others

Evolutionary Algorithms (EAs) are a family of optimization methods inspired on the ideas of Darwin's evolution of species.

They make a robust class of optimization meta- heuristics with applications in a number of fields

An EA has a **population** of evolving individuals, each encoding a solution for the target problem

Each individual can be evaluated by applying a **fitness function** to the solution encoded

New solutions are created by the application of **reproduction operators** to existing individuals – typically crossover and mutation

Stocastic selection, favouring high fitness individuals, is used to select the individuals to be parents

EAs have been applied in clustering by considering the formulation of the k-means approach, i.e., the **fitness function** is the sum-of-squares of distances from the points to the cluster centroids.

Typically, EA individuals encode a set of coordinates for the centroid of the clusters.

Real-valued encoding schemes are used, as well as appropriate crossover and mutation operators

The combination of EAs with local search optimization methods – **Memetic Algorithms** – has also been proposed. In this case the local opt. method is the Lloyd algorithm (k-means).

Self-organizing trees (SOTs) combine SOMs with ideas from divisive hierarchical clustering. The output nodes are organized in binary trees and their number os not fixed.

Quality-based clustering – a number of greedy algorithms that try to find a number of clusters with a guranteed quality, i.e. the members of the cluster are co-expressed.

Bi-clustering algorithms – the aim is to simultaneously group experiments and genes.

Often, the result of microarray data analysis is a list of genes.
• The list has to be summarized with respect to its biological meaning. For this, information about the genes and the related proteins has to be gathered.

PRIMARY DATABASES
Some information about genes and the encoded proteins is available already from sequence (primary) databases, e.g. GenBank. Database accession number, nucleotide and protein sequences, database cross references, and a sequence name that may or may not give a hint to the function.

CURATED DATABASES
In contrast, some databases are *curated*. That means that biologists will get the information first and compare them with literature before it goes into the database. Thus, the database is of high quality, but it takes some time until a newly discovered sequence is entered. Because information is only entered by curators, *annotation* can be unified.
A very famous curated database is SWISSPROT/UniProtKB (http://www.expasy.ch).
There are other organism specific curated databases like ecocyc (www.ecocy.org)

To overcome some of the problems, an annotation system has been created: Gene Ontology (http://www.geneontology.org).
Ontology means here the art (or science) of giving everything its correct name.

- It represents a unified, consistent system, i.e. terms occur only once, and there is a dictionary of allowed words.
- Furthermore, terms are related to each other: the hierarchy goes from very general terms to very detailed ones.

Gene Ontology by itself is only a system for annotating genes and proteins. It does not relate database entries to a special annotation value.

- Luckily, research communities for several model organisms have agreed on entering Gene Ontology information into the databases. As this is done ‘by hand’, GO annotation for most organisms is far from complete.

Bioconductor metadata packages

Runs in R
These packages contain one-to-one and one-to-many mappings for frequently used chips, especially Affymetrix arrays.
• Information available includes gene names, gene symbol, database accession numbers, Gene Ontology function description, enzmye classification number (EC), relations to PubMed abstracts, and others.

Other Tools (Mapping of Gene Expression data in Pathways)
• Some systems collect pathway maps from users. However, most pathway information is still on metabolic pathways (see KEGG for this).
• For other pathway information, see www.biocarta.com and www.stke.org (requires registration). For software, see e.g. GeneMAPP (www.genmapp.org) or GoMiner (http://discover.nci.nih.gov/gominer).

In classification, a number of examples where the class is known (thus **Supervised Learning**) is given – **training examples**

Datasets are usually given by tables where the rows are the examples and the columns are the features of each examples.

outlook	temper.	humidíty	wind	**PLAY**
sunny	32	low	weak	NO
rainy	12	high	strong	NO
overcast	20	low	weak	YES
sunny	24	low	weak	YES
(…)				

An algorithm is used to build a model (e.g decision tree, set of rules, ...) given the training examples

The model **generalizes** the training examples

The model built is capable of predicting the class for new examples (where the class is unknown)

A good algorithm should choose an accurate model but avoid overfitting the training cases

Main use of classification in microarrays:

To **classify experiments** in one of a finite set of alternatives (e.g. healthy, cancer type A or B,, …) given the values if the expression for the genes in that condition.

- **Examples** are the experiments/ conditions

- **Attributes** are the values of the expression for each gene

Or, to classify genes in functional groups; the role of experiments and genes are switched.

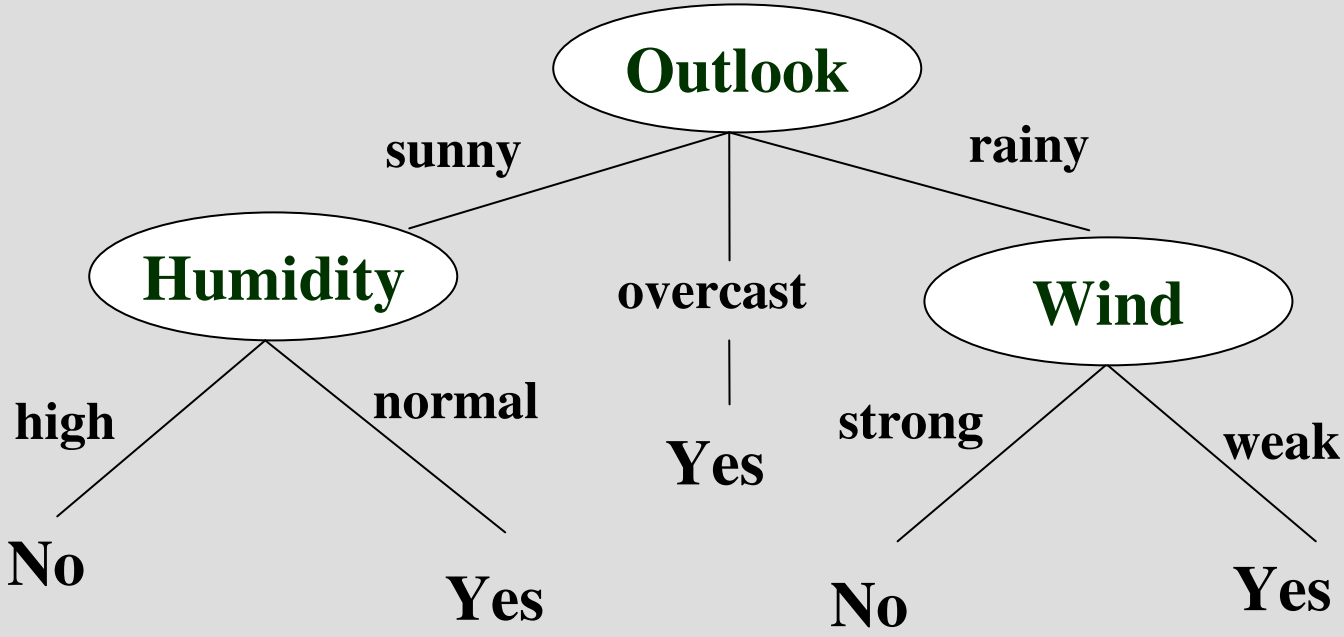
Alternative **models** for classification:

- Decision trees
- Classification rules
- Neural Networks
- Support Vector Machines
- Instance-based learning
- Bayesian models
- ...

Each of these paradigms imposes a given structure. The details / parameters of the model have to be determined, typically using **optimization methods**.

- In **decision trees**, models are n-ary trees, where nodes test the values of attributes and leafs keep the value of the class.

- Process of classifying a new example is recursive, going down the tree and testing the value of the attribute in each node, and following the approprite branch until a leaf is reached.



- Process of **building a decision tree** involves choosing the attributes to test at each level.

- The process is recursive following a top-down approach: starts with all the training examples and finds the best attribute to test – the one that best differentiates the examples in terms of the class (ideas from information theory).

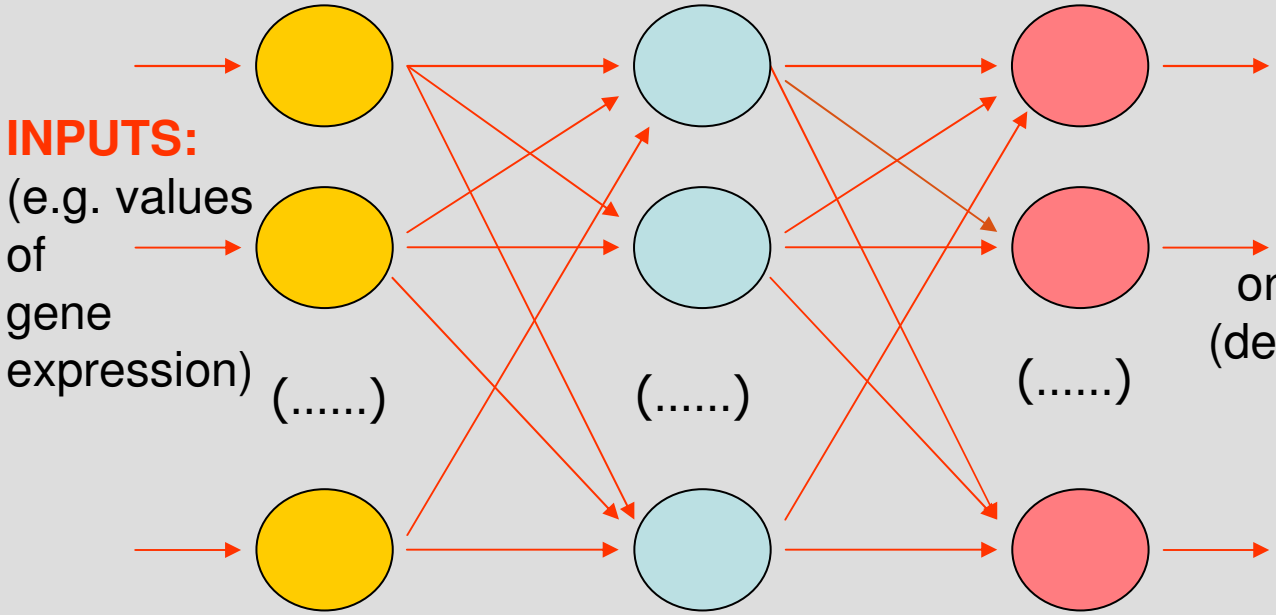
- The process is recursive and it is repeated for each branch, considering only the examples that follow that path.

- Most popular algorithms: ID3 and C4.5

Artificial Neural Networks are models that mimic the information processing in the nervous central system: natural neurons

Able to define nonlinear mappings between a number of input and output variables; quite robust when facing noisy data; used in regression and classification

Most common - **Multilayer Perceptrons** (MLPs)



MLPs “learn“ by setting the weights of the connections in the networks, using numerical optimization methods in order to minimize an error function (e.g. Backpropagation)

The topology of the MLP defines its complexity; more nodes/ weights imply more complex networks

The hardest problem in using MLPs is overfitting the training data; the best way to avoid this problem is to keep the model as simple as possible !!

Support Vector Machines (SVMs) rely on hyperplanes that separate the training examples in a "feature space" of a higher dimensionality.

They make an efficient alternative, very resilient to overfitting the training data since they choose the maximum separation hyperplane

They are quite efficient in training when compared to MLPs; they work fairly well with a large number of attributes

The definition of the kernel functions makes them flexible to define distance functions

They have been applied in microarray classification in work of several researchers

Can also be applied in regression

A model is built using a set of training examples – it is obvious that we cannot use that set of examples to evaluate its performance / compare different models

Two solutions:
- Split the original data into two sets: **training** and **test** set; the first is used to build the model, the second is used to evaluate it.
It has the disadvantage of "wasting" data

- **Cross validation**
To divide the original set into K folds; for each fold i create a model with the training data being all examples not in fold i and evaluate the model with examples from fold i; average the results for the K folds.
Slower but more reliable; uses all data available

Paper by Brown et al (2000) , PNAS

- Dataset: *S. cerevisiae*, nearly 2500 genes, 79 experiments
- Aim: classify genes in 6 functional classes
- Models used: Decision trees, SVMs, linear discriminants
- Each model: 79 attributes; 2500 examples
- Used training/ test set split to evaluate the models.

- Results: SVMs outperformed all other methods

The most important obstacle to using DM classification techniques when working with microarray data is the dimensionality, i.e. the huge number of attributes

Typically the classification algorithms presented before work well with tens of attributes, but tend to degrade their performance with hundreds or thousands, specially when the number of examples is quite smaller

Feature selection techniques are designed to reduce the number of attributes in the model, before the data is presented to the learning algorithm.

There are two major classes of feature selection techniques:

Filter methods

These are general methods that are based in the general features of the data, being independent of the algorithm used in the learning step.
In the case of MAs these are in general useful to perform a first reduction of the number of genes (typically to a few hundred). Sometimes a ranking can be created.

Wrapper methods

These FS methods are embedded into the learning algorithm.
These are used in MAs to further reduce the number of genes to a few tens, in combination with learning algorithms (such as SVMs)
Typically, they involve an optimization method being the cost function given by the accuracy and the number of genes

Deutsch (2003), Bioinformatics

- Datasets: Leukemia dataset (Golub,1999) - >7000 genes and 72 samples, small round blue cell tumors (Khan,2001) - 2300 genes and 80 samples

- In both cases, the purpose is to classify experiments, labeling each with the type of cancer

-Aim of this work: to develop a FS algorithm to reduce the number of genes needed to reach a diagnosis

- Classification algorithm: the simplest, 1 nearest neighbour;

-Filter methods: preprocess the data removing unimportant genes; ranks the genes

- Wrapper method: Evolutionary Algorithm encoding which genes will be in the classifier; fitness function takes accuracy of classification into account

DNA microarrays

Microarrays measure the concentration of mRNA

There is a long way from cell harvest to measuring the transcription level – and the data can be noisy

Proper pre-processing is important

Analysis of microarrays

Several assumptions are made in the progress of analysis

Data analysis can be conducted in different ways:

- Hypothesis testing (t-test), Cluster analysis, Classification

Data analysis should be integrated with existing knowledge from literature and databases

DNA microarrays are a useful tool in Functional Genomics, given proper methods for data analysis

Data Mining techniques can provide a very rich set of solutions in the analysis of microarray data

The methods presented in this session are only an appetizer … there are lots of nice papers out there to read ☺