Machine Learning in Bioinformatics

BMI/CS 776

www.biostat.wisc.edu/bmi776/

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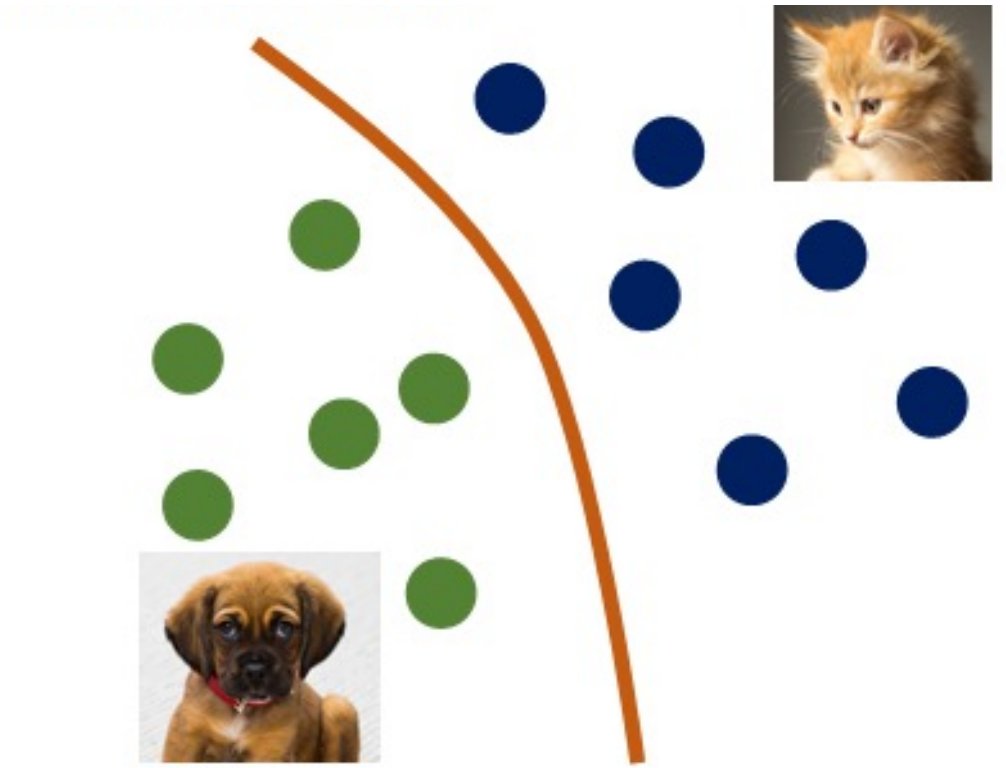
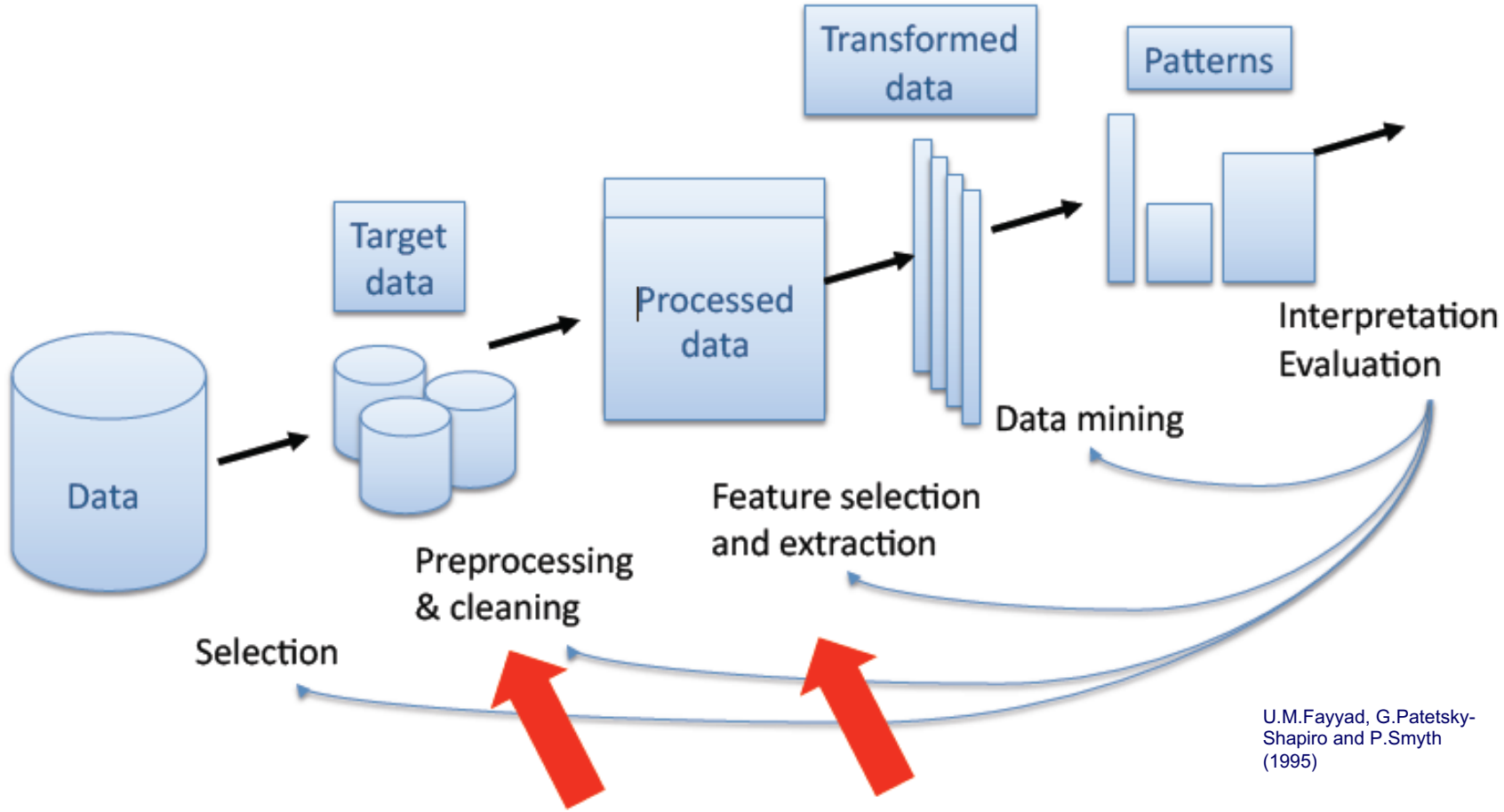
Goals for lecture

- Basic machine learning ideas
- Feature selection
- Unsupervised learning
  - Partitioning vs. hierarchical clustering
- Supervised learning
  - Classification
- Applications in bioinformatics

Dog vs Cat

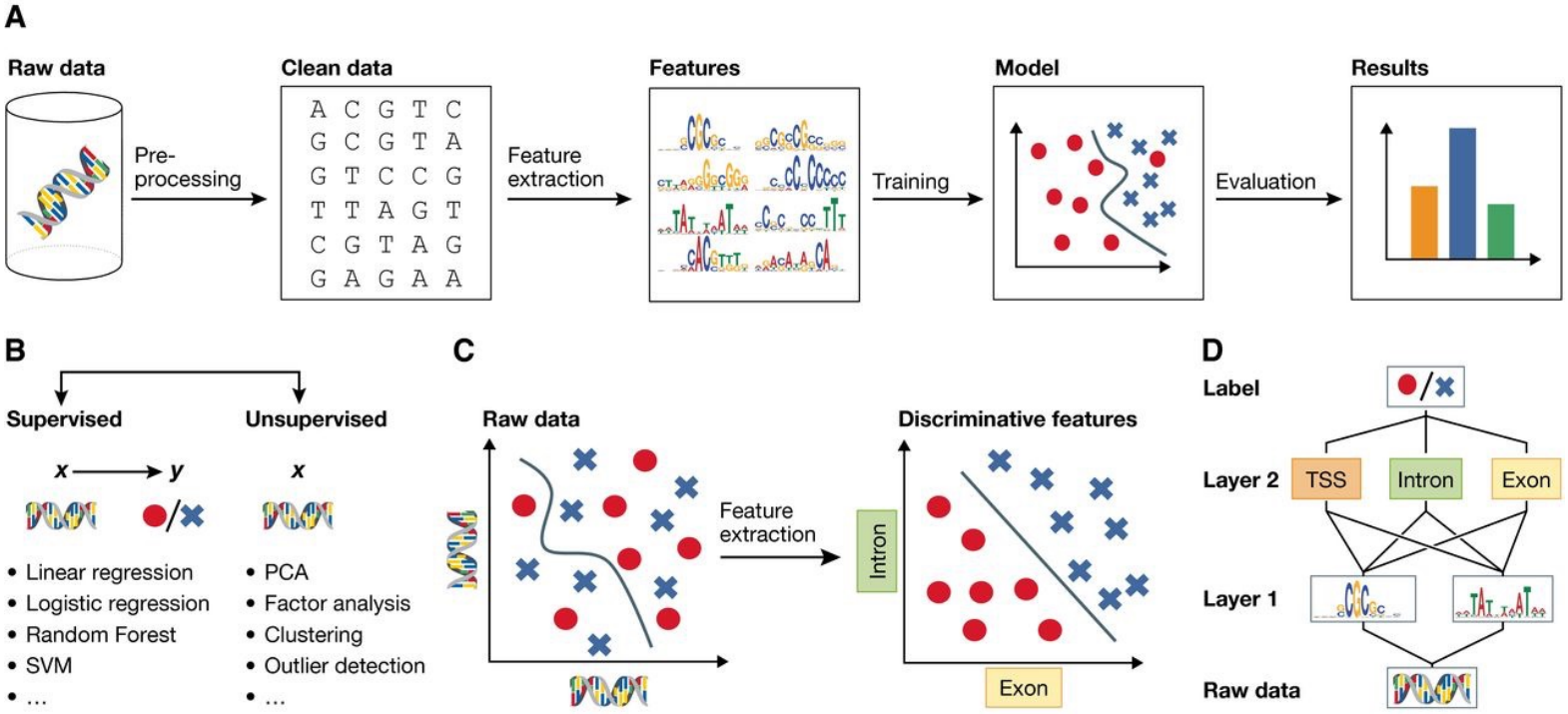


Knowledge Discovery in Databases (KDD)



U.M.Fayyad, G.Patetsky-Shapiro and P.Smyth (1995)

Example: Machine learning in genomics



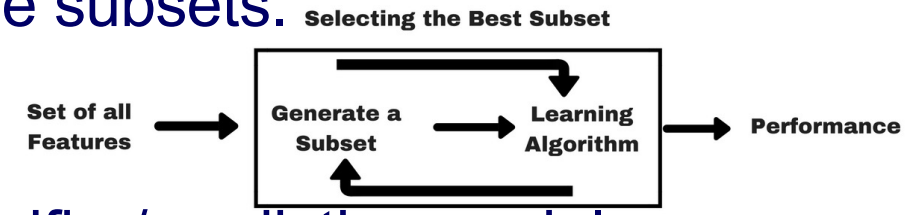
Christof Angermueller et al. Mol Syst Biol 2016;12:878

molecular systems biology

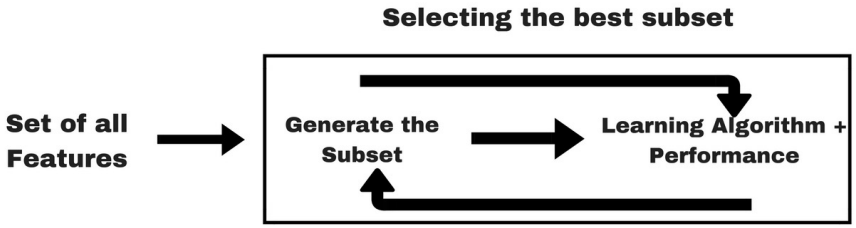
Feature selection

- **Filter approach** scores and ranks features independently of the predictor (classifier).
  - For example, t-test, correlation coefficient

- **Wrapper approach** uses a classifier/predictive model to search (many) best features or feature subsets.
  - Recursive feature elimination



- **Embedded approach** uses a classifier/predictive model to build a (single) model with a subset of features that are internally selected.
  - LASSO regression

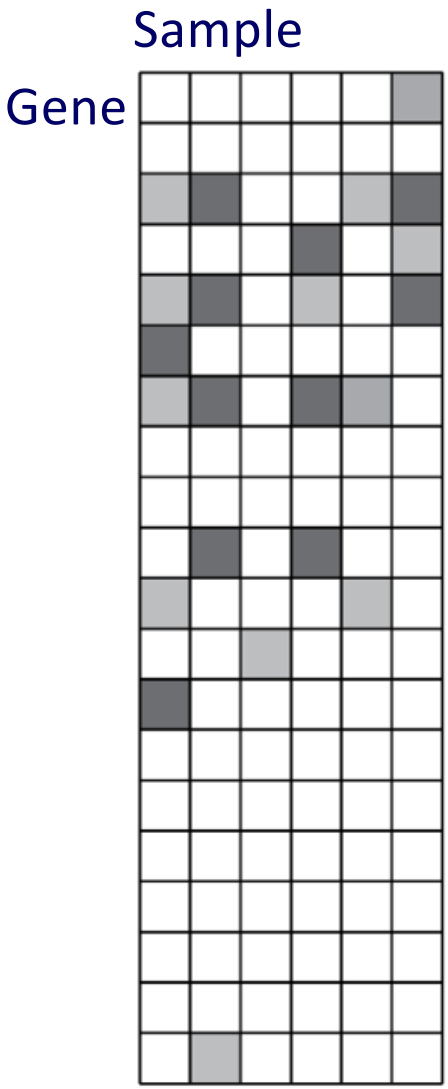


https://www.analyticsvidhya.com/blog/2016/12/introduction-to-feature-selection-methods-with-an-example-or-how-to-select-the-right-variables/

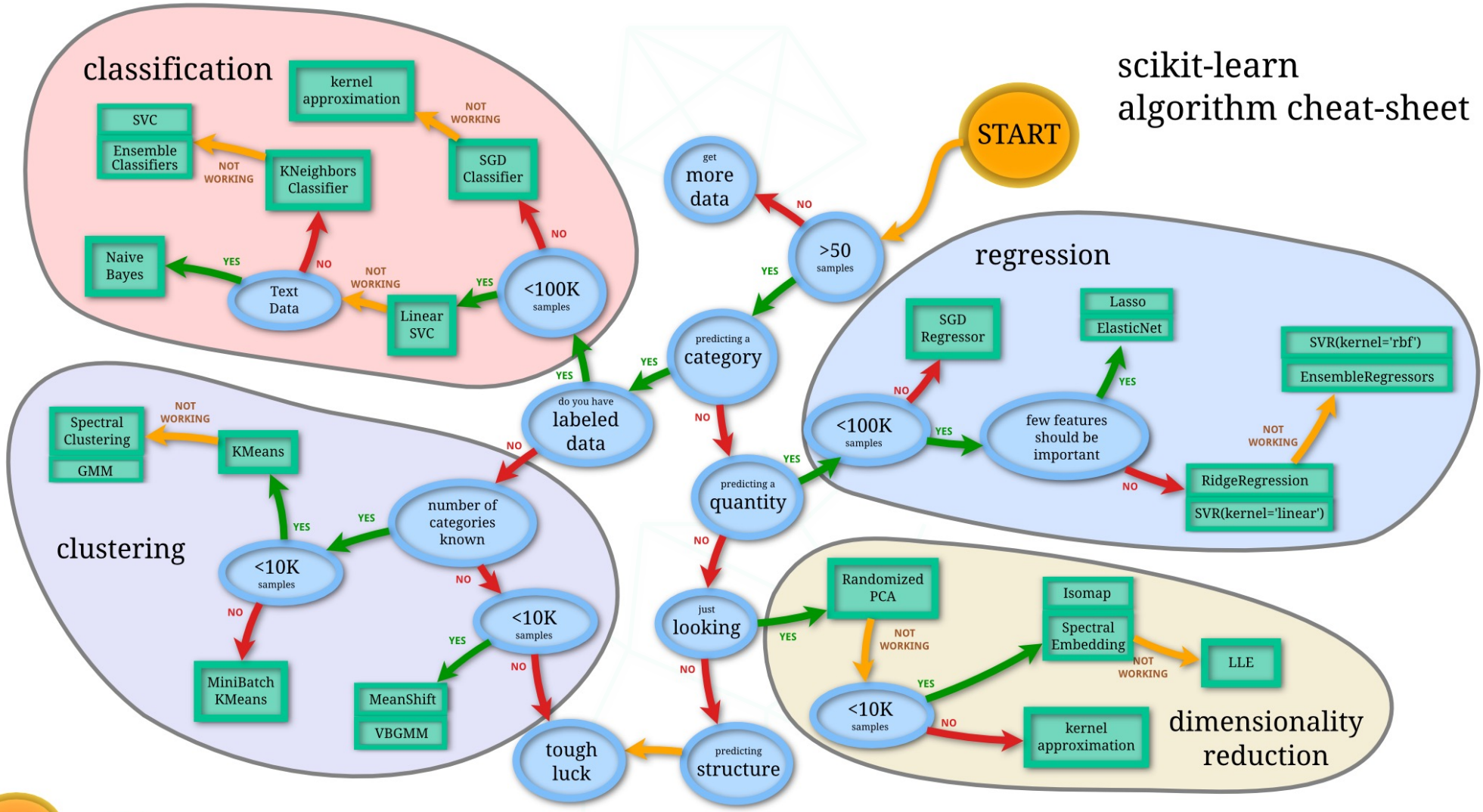
Differentially expressed genes

- Identify genes with different levels in two conditions
- Examples
  - Highly expressed genes in cancer cells vs. health cells
- Filter method for selecting “feature” genes

What can we learn from a data matrix?



The World of Machine Learning



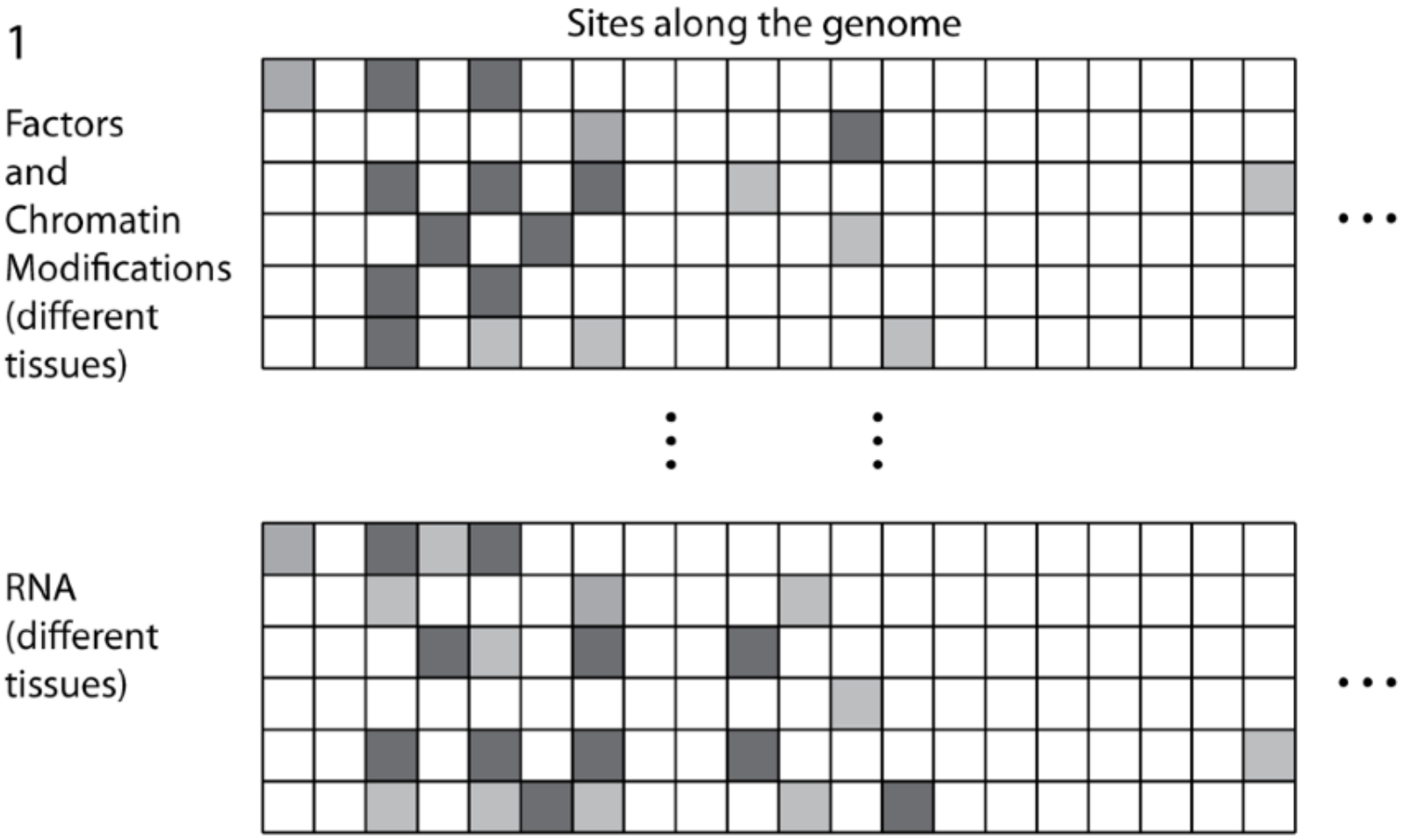
Back

SciKit learn: http://scikit-learn.org/stable/tutorial/machine_learning_map/

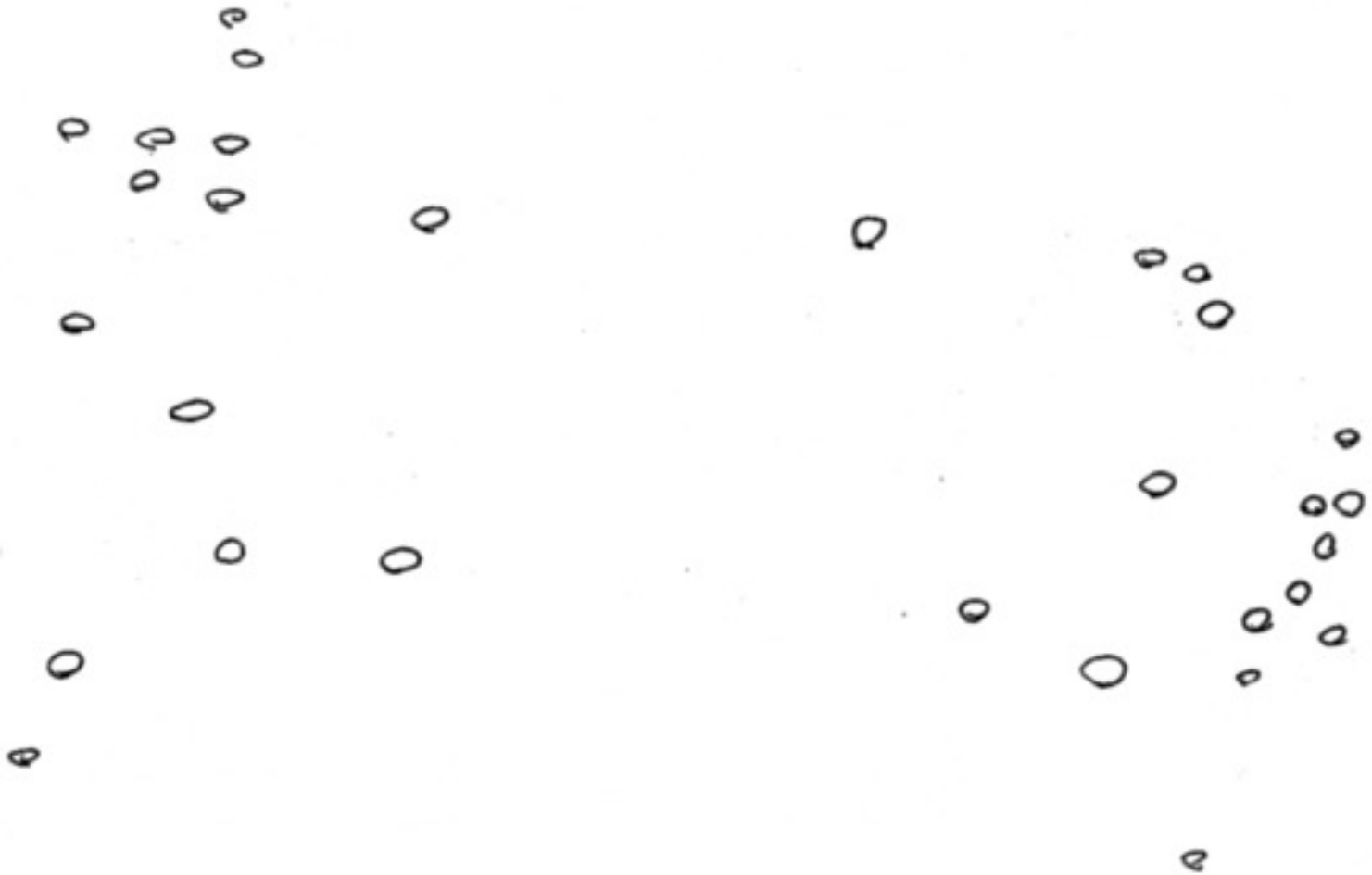
Unsupervised learning

- Partitioning clustering
  - K-means
  - K-medoids
- Hierarchical clustering

Structure of Genomic Features Matrix

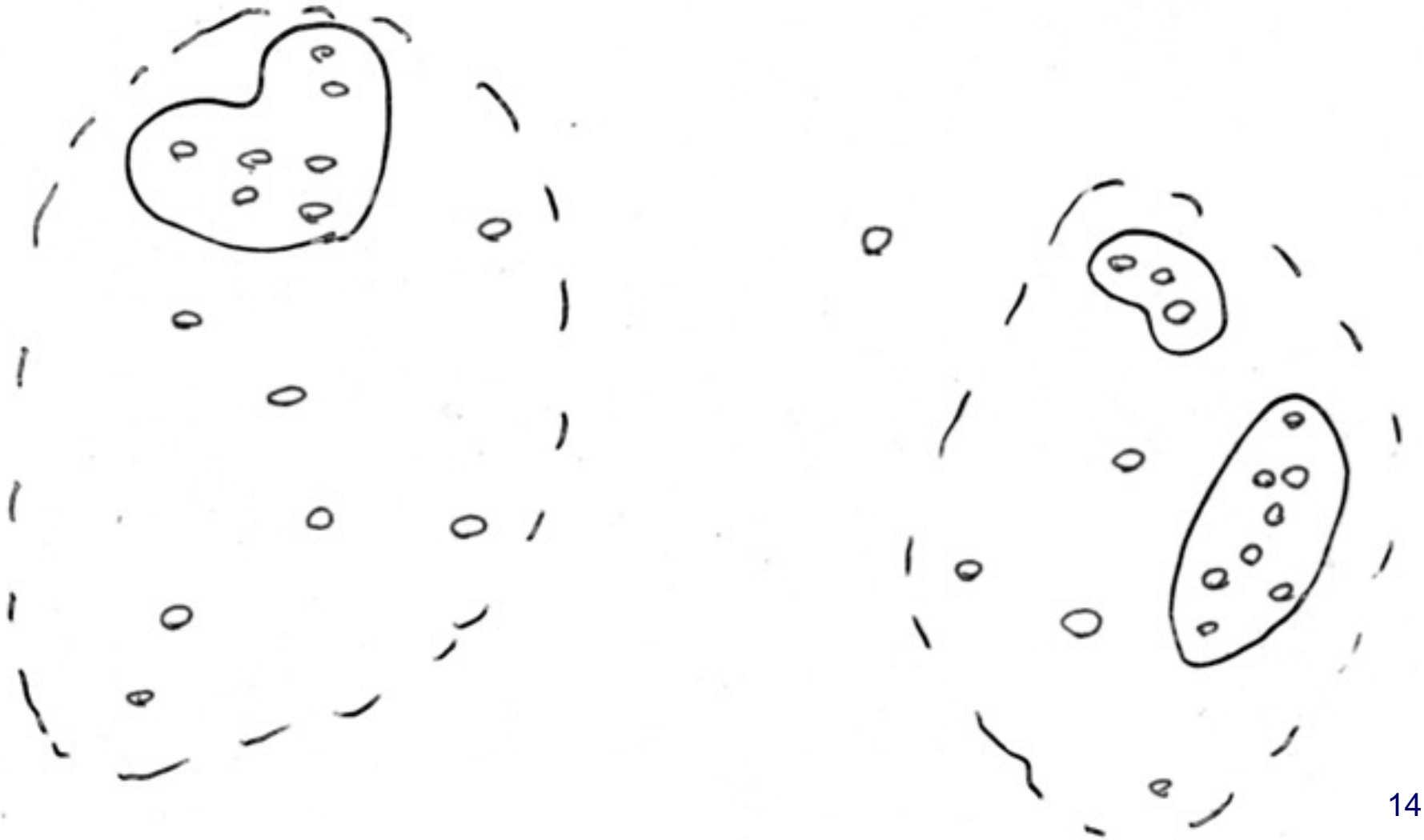


Represent predictors in abstract high dimensional space

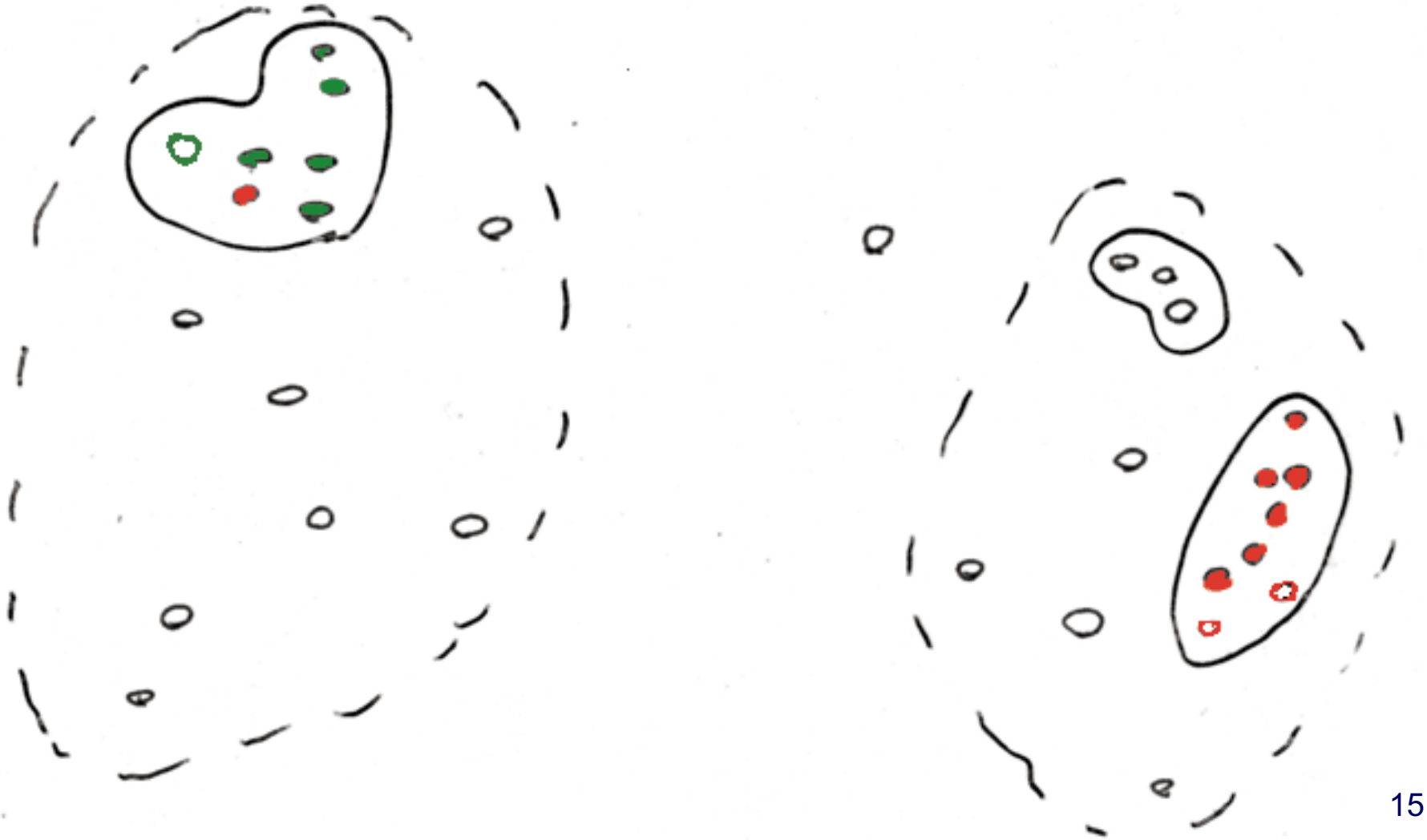


“Label” Certain Points

“Cluster” predictors (Unsupervised)

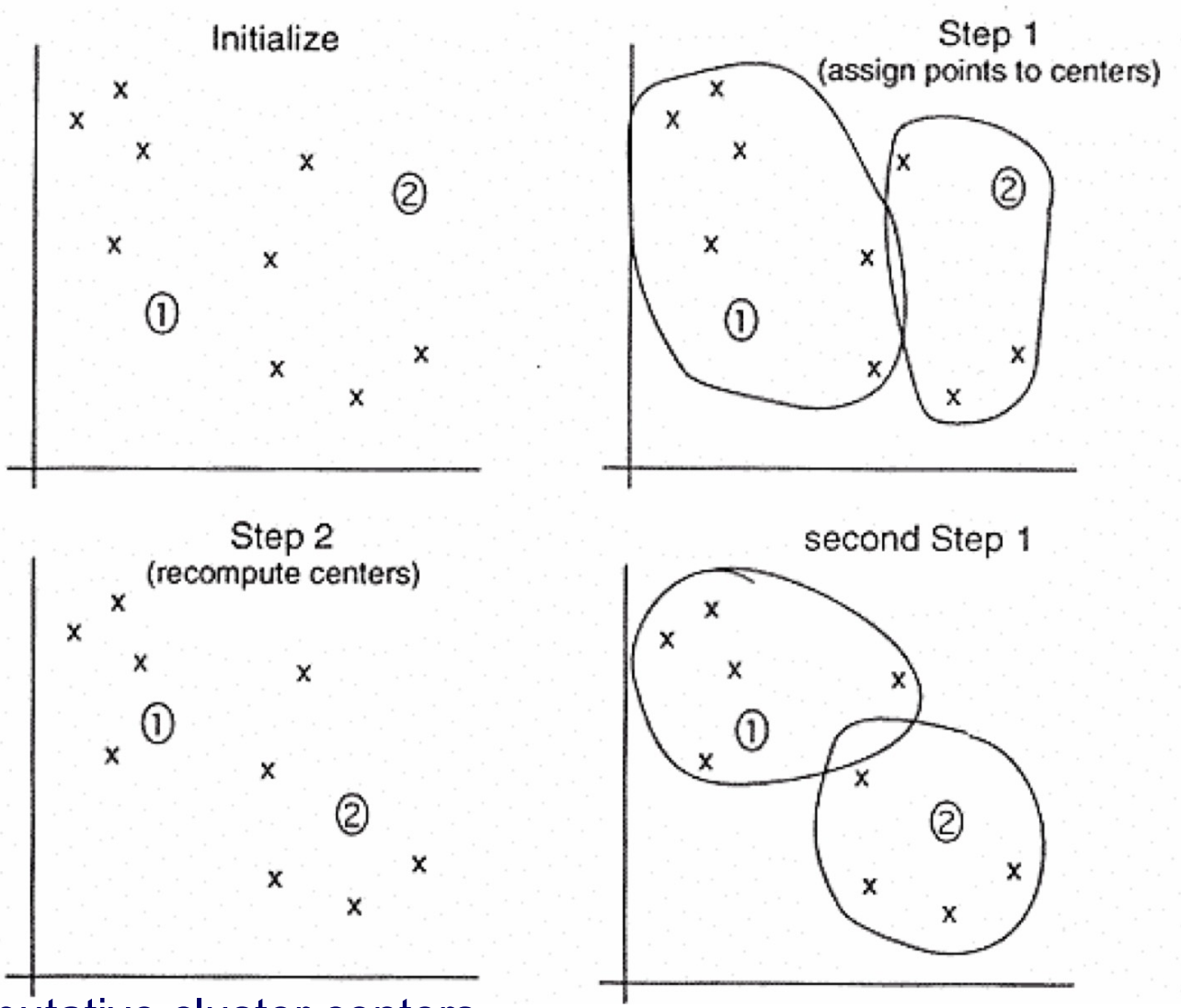


Use Clusters to predict Response
(Unsupervised, guilt-by-association)



For example, PCA coefficients over PC1 and PC2

K-means



1) Pick *K* random points as putative cluster centers.
2) Group the points to be clustered by the center to which they are closest.
3) Then take the mean of each group and repeat, with the means now at the cluster center.
4)Stop when the centers stop moving.

K-means: Setup

- $x_1, \ldots, x_N$ are data points or vectors of observations
- Each observation (vector x_i) will be assigned to one and only one cluster
- $C(i)$ denotes cluster number for the i^{th} observation
- Dissimilarity measure: Euclidean distance metric
- *K*-means minimizes within-cluster point scatter:

$$W(C) = \frac{1}{2}\sum_{k=1}^{K}\sum_{C(i)=k}\sum_{C(j)=k}\|x_i - x_j\|^2 = \sum_{k=1}^{K} N_k \sum_{C(i)=k}\|x_i - m_k\|^2$$

where

m_k is the mean vector of the k^{th} cluster

N_k is the number of observations in k^{th} cluster

Within and Between Cluster Criteria

Let's consider total point scatter for a set of *N* data points:

$$T = \frac{1}{2}\sum_{i=1}^{N}\sum_{j=1}^{N} d(x_i, x_j)$$

Distance between two points

T can be re-written as:

$$T = \frac{1}{2}\sum_{k=1}^{K}\sum_{C(i)=k}\left(\sum_{C(j)=k} d(x_i, x_j) + \sum_{C(j)\neq k} d(x_i, x_j)\right)$$
$$= W(C) + B(C)$$

Where,

$$W(C) = \frac{1}{2}\sum_{k=1}^{K}\sum_{C(i)=k}\sum_{C(j)=k} d(x_i, x_j)$$

Within cluster scatter

$$B(C) = \frac{1}{2}\sum_{k=1}^{K}\sum_{C(i)=k}\sum_{C(j)\neq k} d(x_i, x_j)$$

Between cluster scatter

If *d* is square Euclidean distance, then

$$W(C) = \sum_{k=1}^{K} N_k \sum_{C(i)=k} \|x_i - m_k\|^2$$

and $$B(C) = \sum_{k=1}^{K} N_k \|m_k - m\|^2$$

Grand mean

Minimizing *W*(*C*) is equivalent to maximizing *B*(*C*)

K-means Algorithm

- For a given cluster assignment *C* of the data points, compute the cluster means m_k:

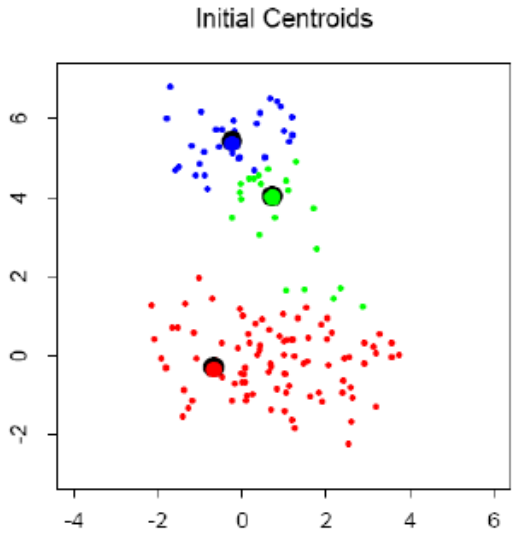
$$m_k = \frac{\sum_{i:C(i)=k} x_i}{N_k},\ k = 1, \ldots, K.$$

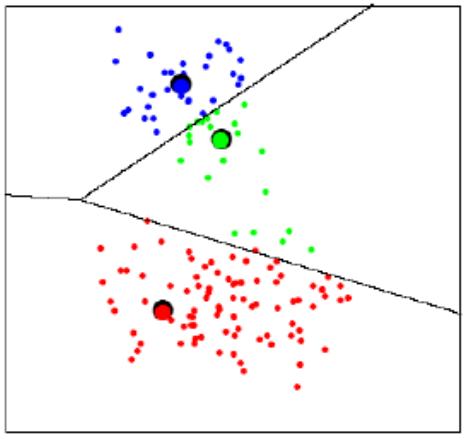
- For a current set of cluster means, assign each observation as:

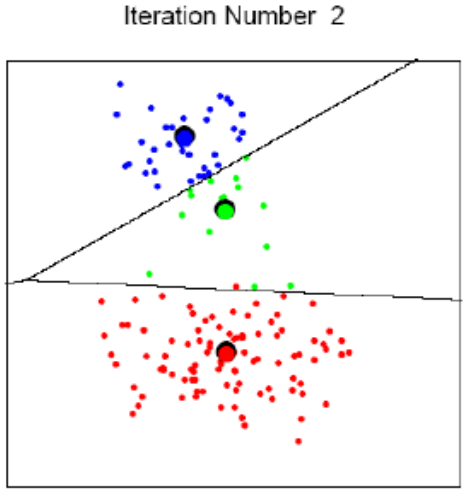
$$C(i) = \arg\min_{1 \le k \le K} \|x_i - m_k\|^2,\ i = 1, \ldots, N$$

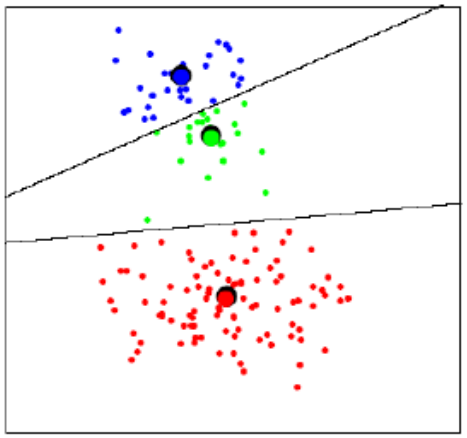
- Iterate above two steps until convergence

K-means clustering example









K-means: summary

- Algorithmically, very simple to implement
- *K*-means converges, but it finds a local minimum of the cost function
- Works only for numerical observations
- *K* is a user input; alternatively BIC (Bayesian information criterion) or MDL (minimum description length) can be used to estimate *K*
- **Outliers can considerable trouble to *K*-means**

K-medoids Clustering

- *K*-means is appropriate when we can work with Euclidean distances
- Thus, *K*-means can work only with numerical, quantitative variable types
- Euclidean distances do not work well in at least two situations
  - Some variables are categorical
  - Outliers can be potential threats
- A general version of *K*-means algorithm called *K*-medoids can work with any distance measure
- *K*-medoids clustering is computationally more intensive

K-medoids Algorithm

- Step 1: For a given cluster assignment *C*, find the observation in the cluster minimizing the total distance to other points in that cluster:

$$i_k^* = \underset{\{i:C(i)=k\}}{\arg\min} \sum_{C(j)=k} d(x_i, x_j).$$

- Step 2: Assign $m_k = x_{i_k^*},\ k = 1,2,\ldots,K$

- Step 3: Given a set of cluster centers $\{m_1, \ldots, m_K\}$, minimize the total error by assigning each observation to the closest (current) cluster center:

$$C(i) = \underset{1\le k\le K}{\arg\min}\, d(x_i, m_k),\ i = 1,\ldots,N$$

- Iterate steps 1 to 3

K-medoids Summary

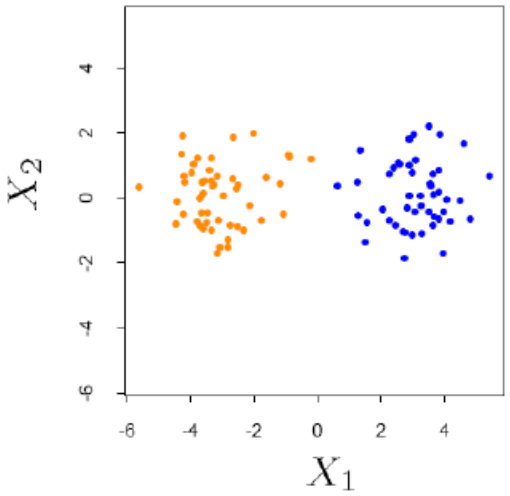
- Generalized *K*-means
- Computationally much costlier that *K*-means
- Apply when dealing with categorical data
- Apply when data points are not available, but only pair-wise distances are available
  - Kernel functions
- Converges to local minimum

Choice of *K*?

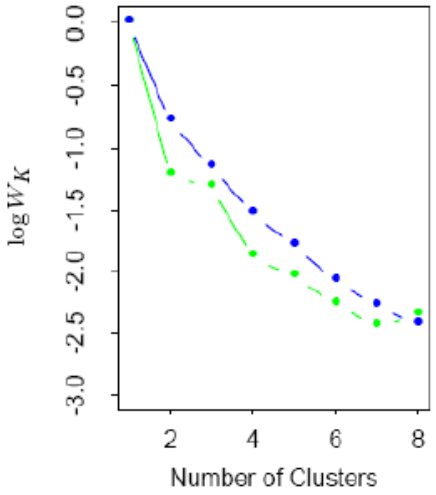
- Can $W_K(C)$, *i.e.*, the within cluster distance as a function of *K* serve as any indicator?
- Note that $W_K(C)$ decreases monotonically with increasing *K.* That is the within cluster scatter decreases with increasing centroids.
- Instead look for gap statistics (successive difference between $W_K(C)$):

$$\{W_K - W_{K+1} : K < K^*\} >> \{W_K - W_{K+1} : K \geq K^*\}$$

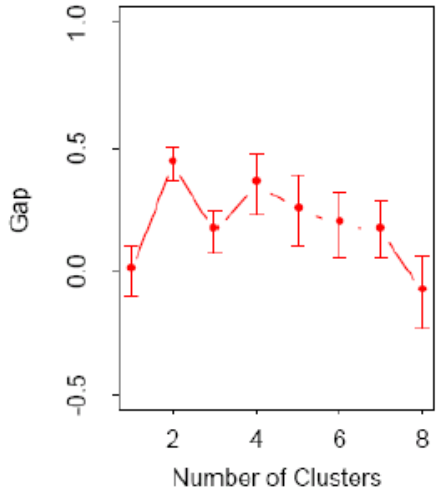
Choice of *K*…



Data points simulated from two pdfs



Log(W_K) curve

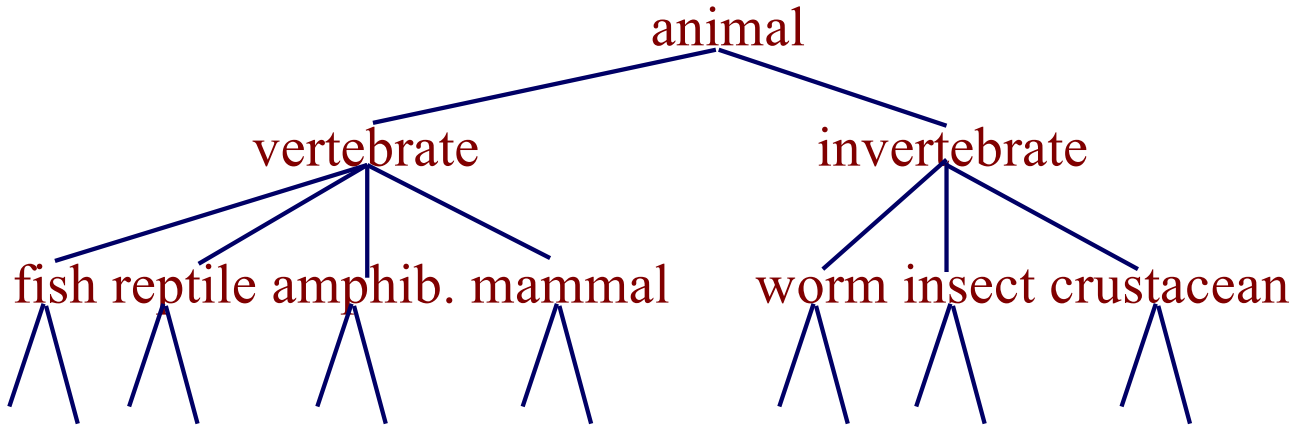


Gap curve

This is essentially a visual heuristic

Hierarchical Clustering

- Build a tree-based hierarchical taxonomy (*dendrogram*) from a set of documents.



How could you do this with K-means?

Hierarchical Clustering

- **Agglomerative (bottom-up):**
  - Start with each gene being a single cluster.
  - Eventually all genes belong to the same cluster.
- **Divisive (top-down):**
  - Start with all genes belong to the same cluster.
  - Eventually each gene forms a cluster on its own.
  - Could be a recursive application of K-means like algorithms
- Does not require the number of clusters *K* in advance
- Needs a termination/readout condition

Hierarchical Agglomerative Clustering (HAC)

- Start with each gene in a separate cluster
  - then repeatedly joins the *closest pair* of clusters, until there is only one cluster.
- The history of merging forms a tree or hierarchy.

How to measure distance of clusters??

Distance Metrics

- properties of metrics

$$\text{dist}(x_i, x_j) \geq 0 \qquad \text{(non-negativity)}$$

$$\text{dist}(x_i, x_j) = 0 \text{ if and only if } x_i = x_j \qquad \text{(identity)}$$

$$\text{dist}(x_i, x_j) = \text{dist}(x_j, x_i) \qquad \text{(symmetry)}$$

$$\text{dist}(x_i, x_j) \leq \text{dist}(x_i, x_k) + \text{dist}(x_k, x_j) \qquad \text{(triangle inequality)}$$

- some distance metrics

Manhattan $$\text{dist}(x_i, x_j) = \sum_e \left| x_{i,e} - x_{j,e} \right|$$

Euclidean $$\text{dist}(x_i, x_j) = \sqrt{\sum_e \left(x_{i,e} - x_{j,e} \right)^2}$$

e ranges over the individual measurements for x_i and x_j

Correlation distance

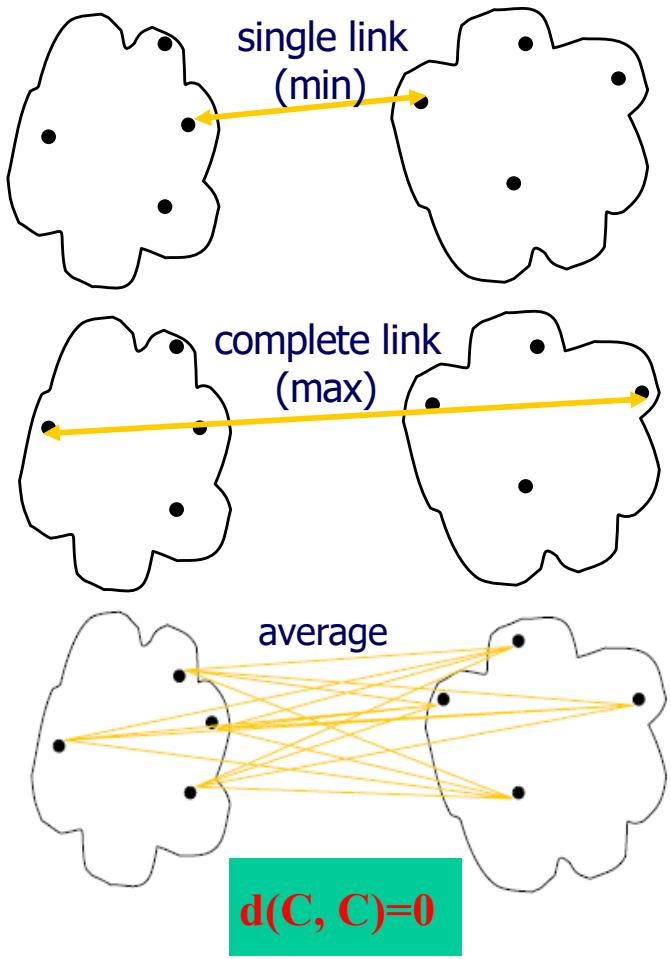
- Correlation distance

$$r_{xy} = \frac{Cov(X,Y)}{\sqrt{(Var(X) \cdot Var(Y)}}$$

  - Cov(X,Y) stands for covariance of X and Y
    - degree to which two different variables are related
  - Var(X) stands for variance of X
    - measurement of a sample differ from their mean

Cluster Distance Measures

- Single link: smallest distance between an element in one cluster and an element in the other, i.e., $d(C_i, C_j) = \min\{d(x_{ip}, x_{jq})\}$
- Complete link: largest distance between an element in one cluster and an element in the other, i.e., $d(C_i, C_j) = \max\{d(x_{ip}, x_{jq})\}$
- Average: avg distance between elements in one cluster and elements in the other, i.e., $d(C_i, C_j) = \text{avg}\{d(x_{ip}, x_{jq})\}$



Machine learning : a probabilistic perspective, Kevin P. Murphy
COMP24111 Machine Learning, Univ. of Manchester

Cluster Distance Measures

Example: Given a data set of five objects characterized by a single continuous feature, assume that there are two clusters: C_1: {a, b} and C_2: {c, d, e}.

	a	b	c	d	e
Feature	1	2	4	5	6

1. Calculate the distance matrix.

	a	b	c	d	e
a	0	1	3	4	5
b	1	0	2	3	4
c	3	2	0	1	2
d	4	3	1	0	1
e	5	4	2	1	0

2. Calculate three cluster distances between C_1 and C_2.

Single link

$$\begin{aligned}\text{dist}(C_1, C_2) &= \min\{d(a,c), d(a,d), d(a,e), d(b,c), d(b,d), d(b,e)\} \\ &= \min\{3, 4, 5, 2, 3, 4\} = 2\end{aligned}$$

Complete link

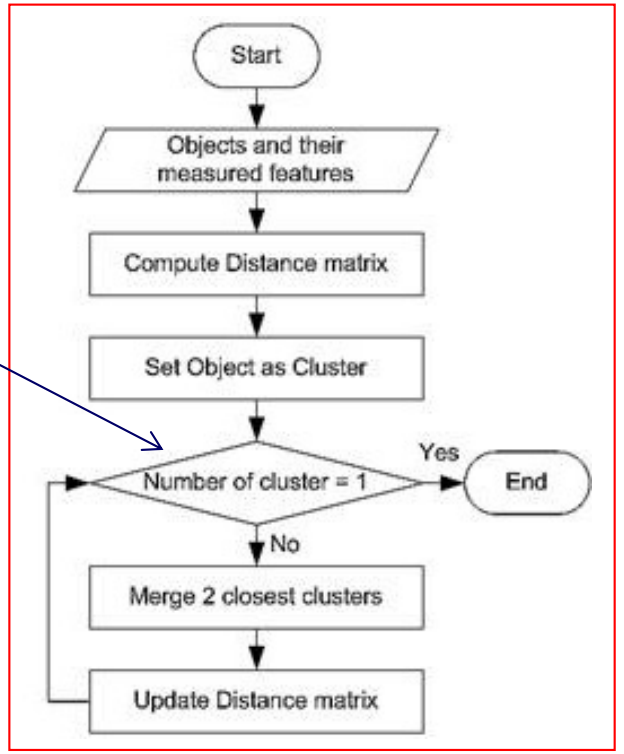
$$\begin{aligned}\text{dist}(C_1, C_2) &= \max\{d(a,c), d(a,d), d(a,e), d(b,c), d(b,d), d(b,e)\} \\ &= \max\{3, 4, 5, 2, 3, 4\} = 5\end{aligned}$$

Average

$$\begin{aligned}\text{dist}(C_1, C_2) &= \frac{d(a,c) + d(a,d) + d(a,e) + d(b,c) + d(b,d) + d(b,e)}{6} \\ &= \frac{3+4+5+2+3+4}{6} = \frac{21}{6} = 3.5\end{aligned}$$

Agglomerative Algorithm

- Convert all features (e.g., genes) into a distance matrix
- Set each gene as a cluster (N genes -> N clusters at the beginning)
- Repeat until number of cluster (or known # of clusters)
  - Merge two closest clusters
  - Update “distance matrix”



Bottom-Up Hierarchical Clustering

given: a set $X = \{x_1 \ldots x_n\}$ of instances

for $i := 1$ to n do

  $c_i := \{x_i\}$ // each object is initially its own cluster, and a leaf in tree

$C := \{c_1 \ldots c_n\}$

$j := n$

while $|C| > 1$

  $j := j + 1$

  $(c_a, c_b) := \underset{(c_u, c_v)}{\text{argmin}}\ \text{dist}(c_u, c_v)$ // find least distant pair in C

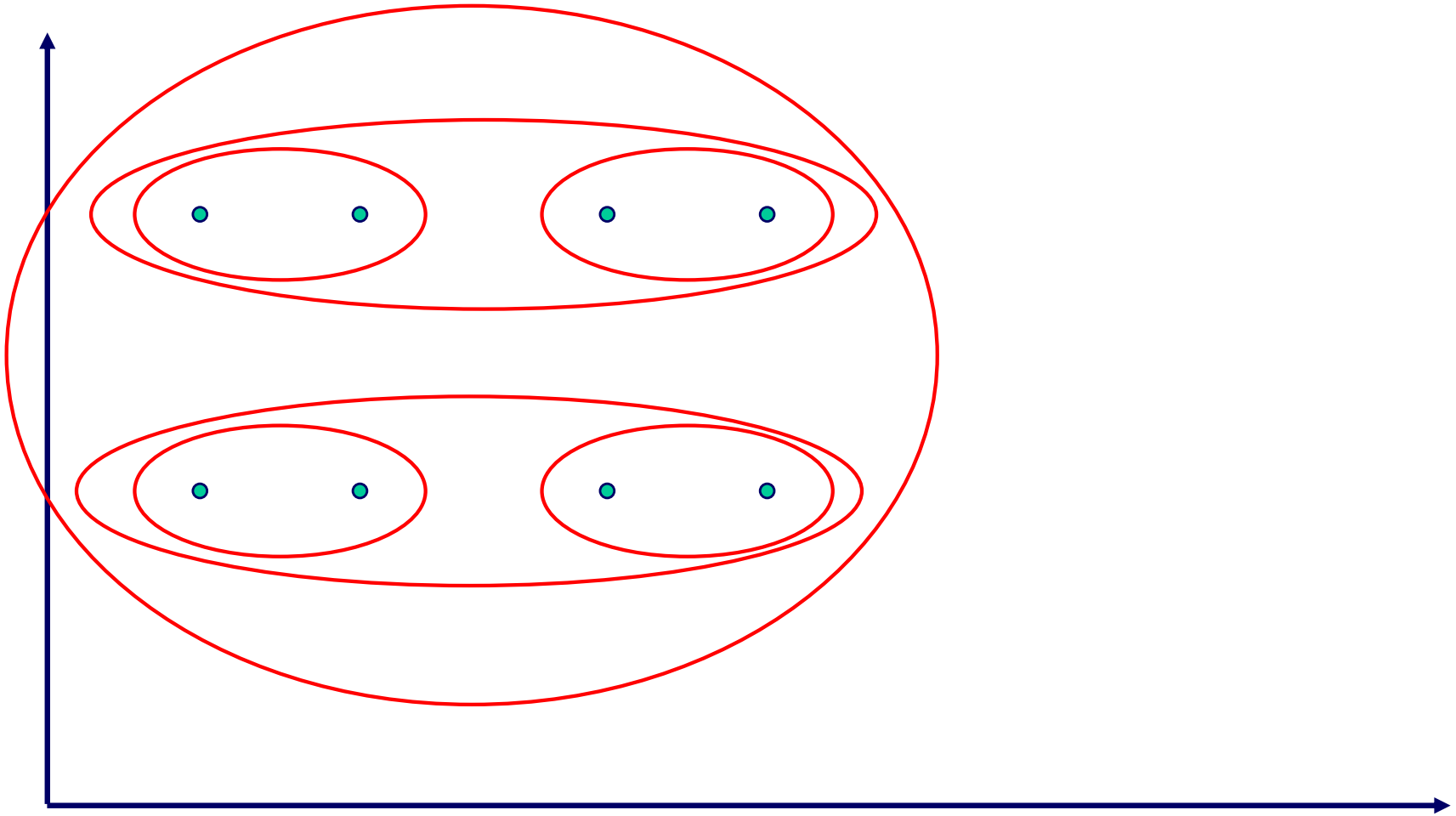
  $c_j = c_a \cup c_b$ // create a new cluster for pair

  add a new node j to the tree joining a and b

  $C := C - \{c_a, c_b\} \cup \{c_j\}$

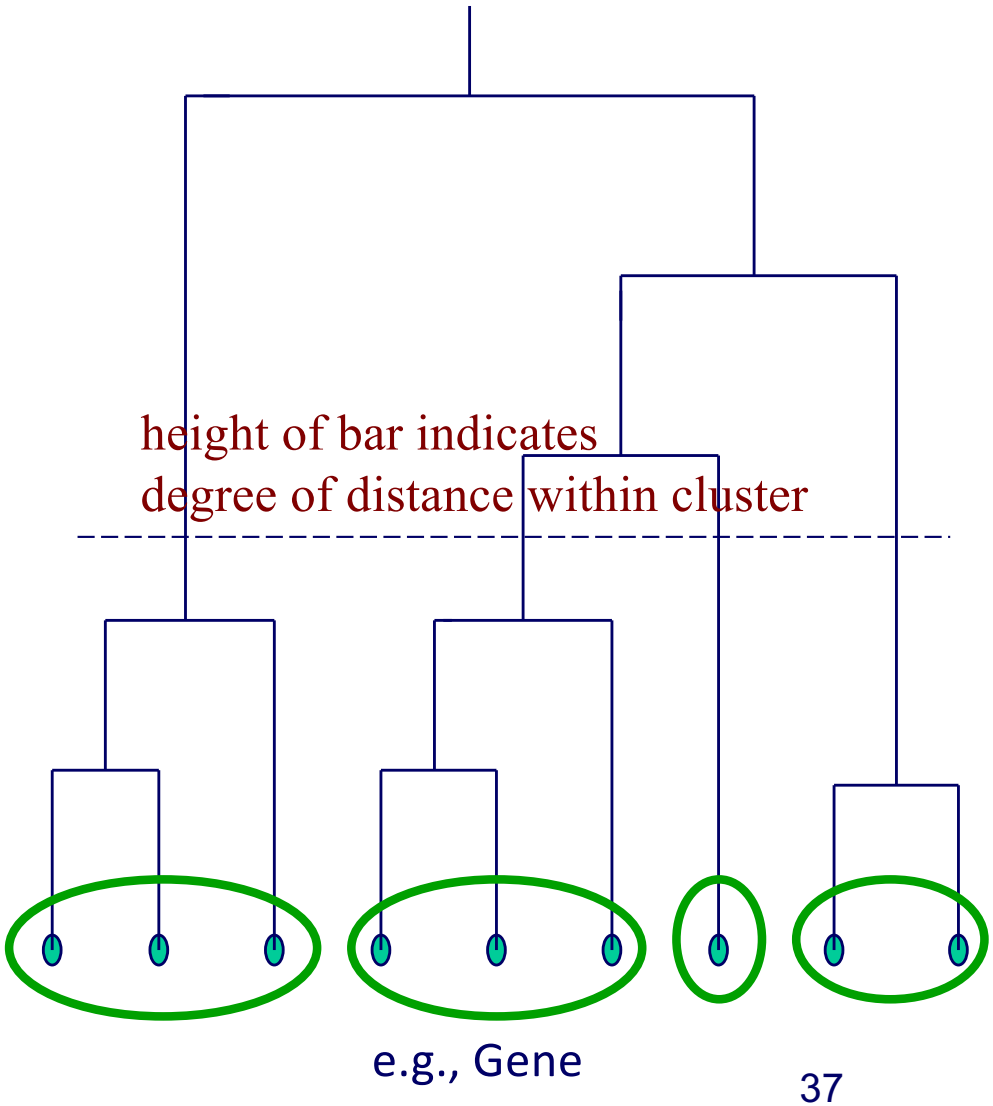
return tree with root node j

Single Link Example

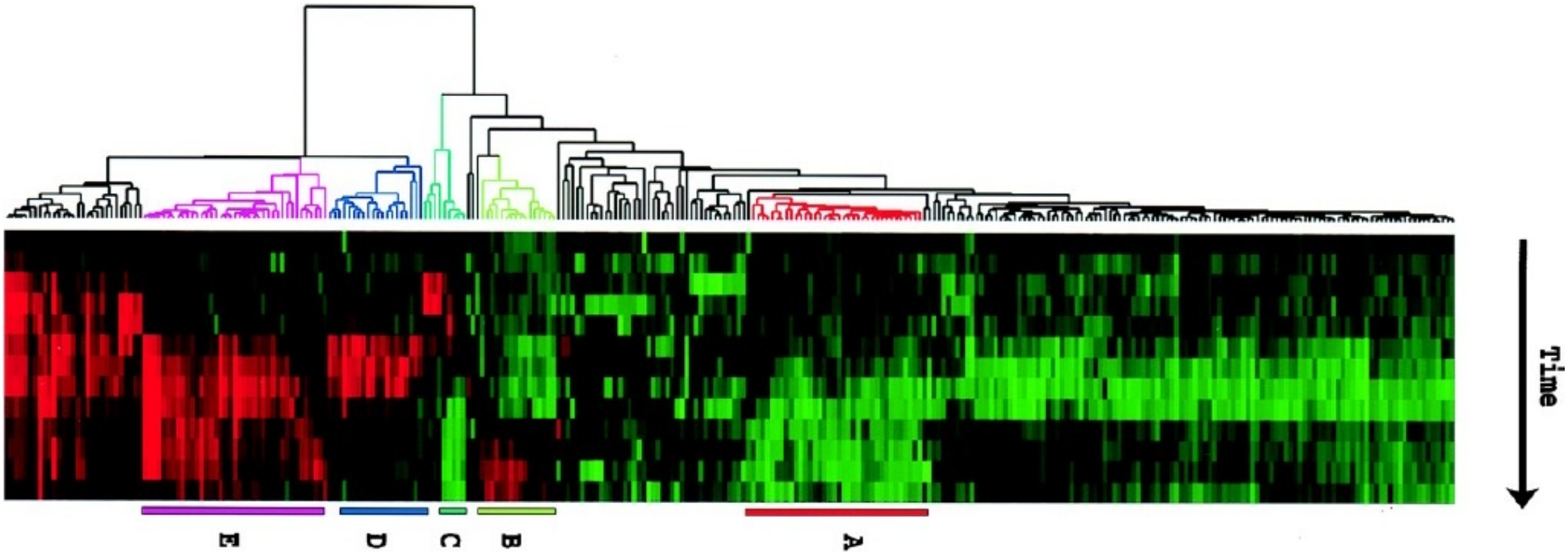


Dendogram: Hierarchical Clustering

- Clustering obtained by cutting the dendrogram at a desired level: each **connected** component forms a cluster.



Hierarchical Clustering of Expression Data



http://www.pnas.org/content/95/25/14863.full

Partitioning or Hierarchical?

Partitioning:

- Advantages
  - Optimal for certain criteria.
  - Genes automatically assigned to clusters
- Disadvantages
  - Need initial k;
  - Often slow computation.
  - All genes are forced into a cluster.

Hierarchical

- Advantages
  - Faster computation.
  - Visual.
- Disadvantages
  - Unrelated genes are eventually joined
  - Rigid, cannot correct later for erroneous decisions made earlier.
  - Hard to define clusters.

Reading list

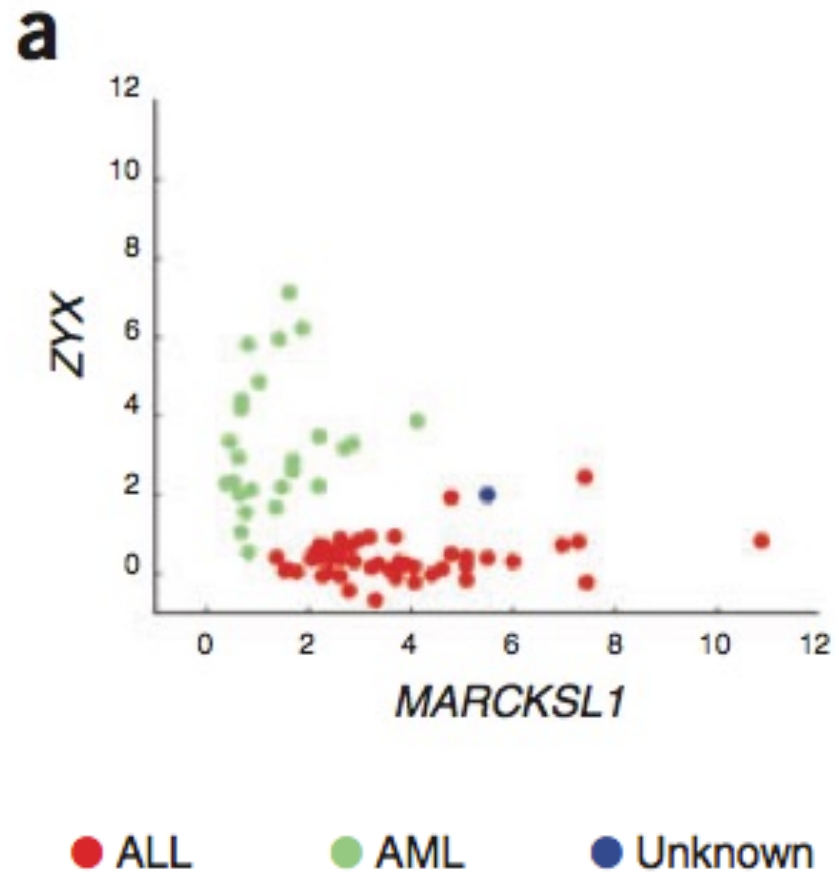
- A. K. Jain and M. N. Murty and P. J. Flynn, Data clustering: a review, ACM Computing Surveys, 31:3, pp. 264 - 323, 1999.
- T. R. Golub et. al, Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring, Science, 286:5439, pp. 531 – 537, 1999.
- Gasch,A.P. and Eisen,M.B. (2002) Exploring the conditional coregulation of yeast gene expression through fuzzy *k*-means clustering. *Genome Biol.*, **3**, 1–22.
- M. Eisen et. al, Cluster Analysis and Display of Genome-Wide Expression Patterns. *Proc Natl Acad Sci U S A* 95, 14863-8, 1998.

Support Vector Machines

- A very powerful tool for classifications
- Example Applications:
  - Text categorization
  - Image classification
  - Spam email recognition, etc

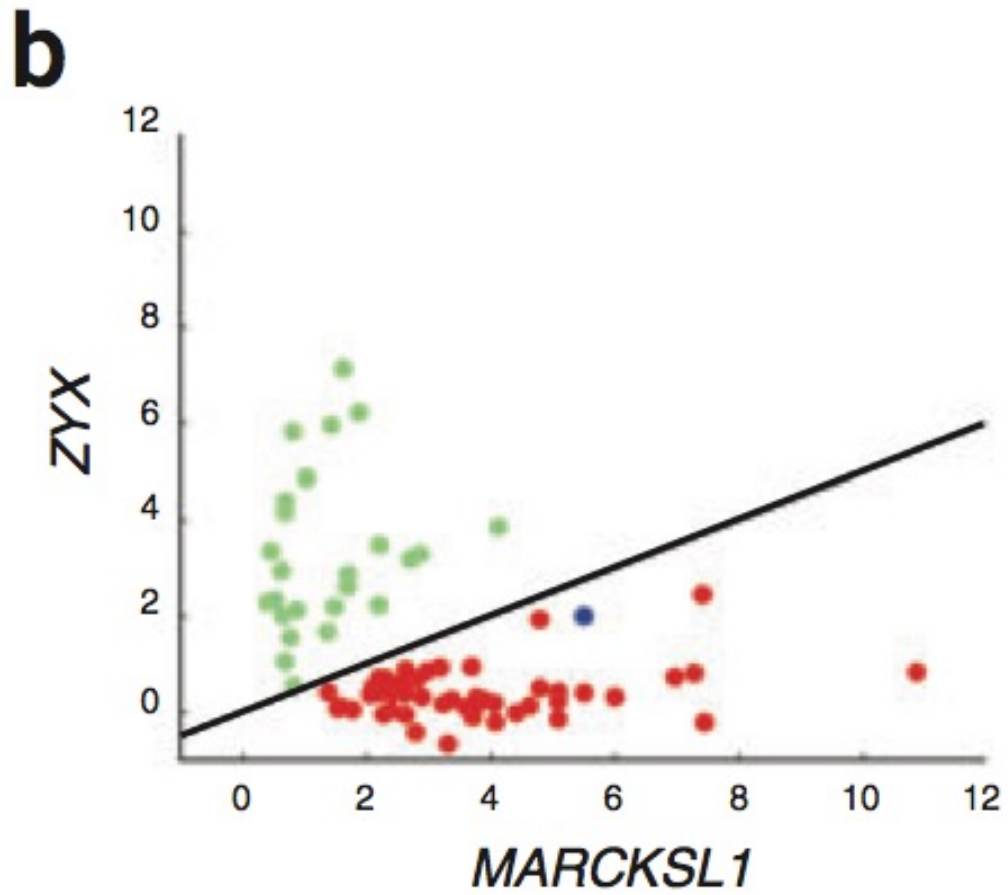
- It has also been successfully applied in many biological problems:
  - Disease diagnosis
  - Automatic genome functional annotation
  - Prediction of protein-protein interactions
  - and more…

- Example: Leukemia patient classification



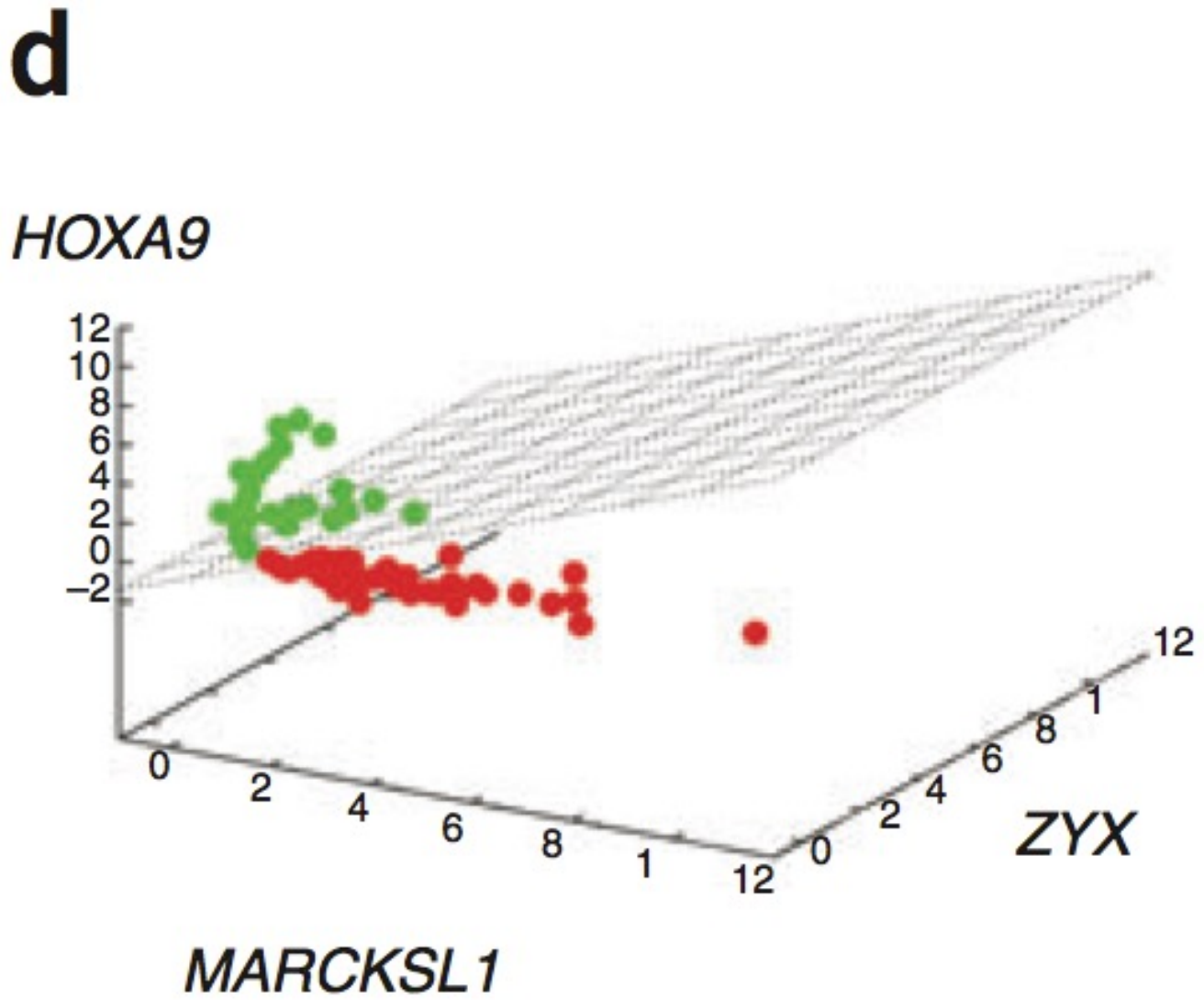
ALL: acute lymphoblastic leukemia
AML: acute myeloid leukemia

William S Nobel. What is a support vector machine? Nature Biotechnology. 2006



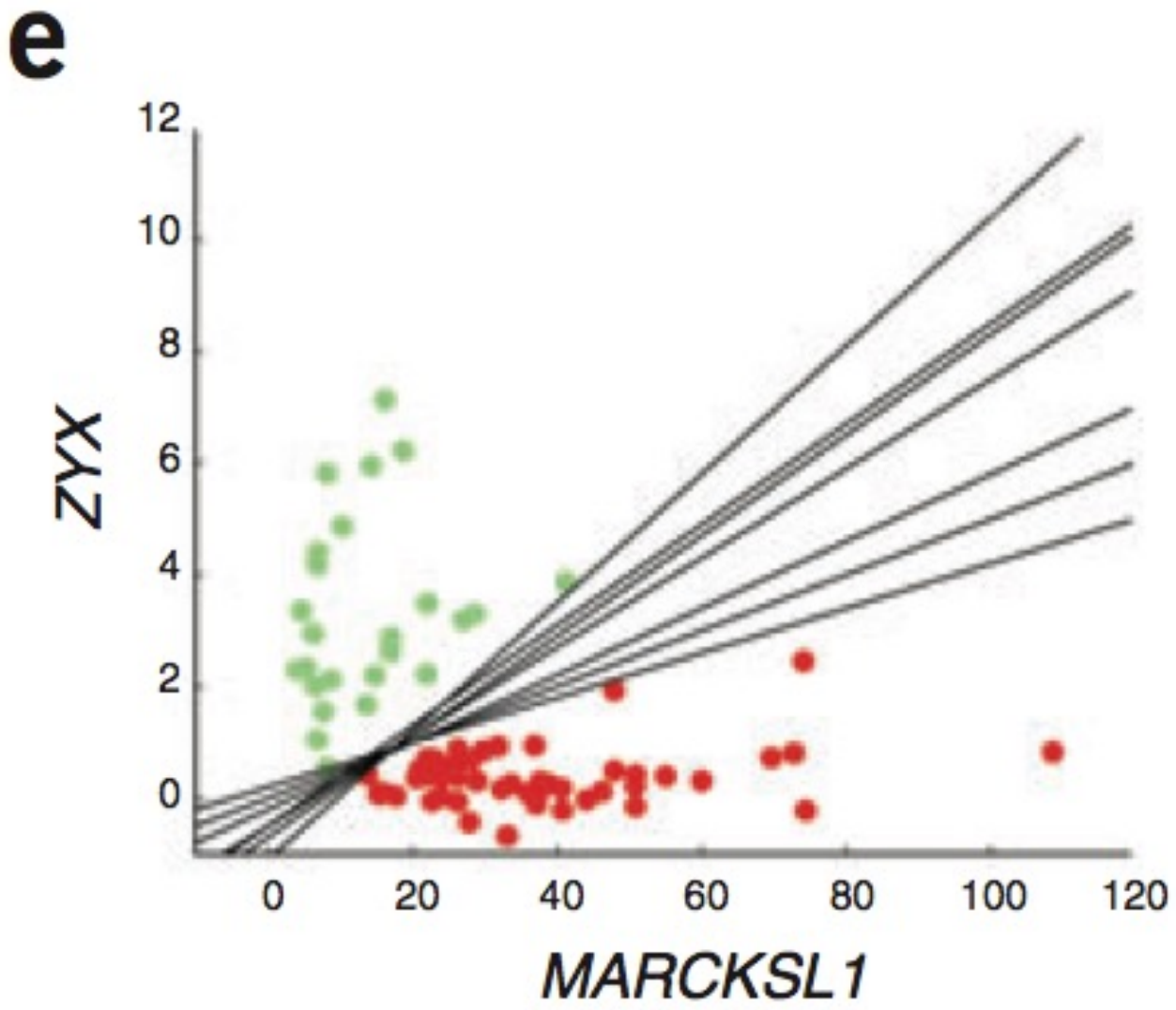
- A simple line suffices to separate the expression profiles of ALL and AML

William S Nobel. What is a support vector machine? Nature Biotechnology. 2006



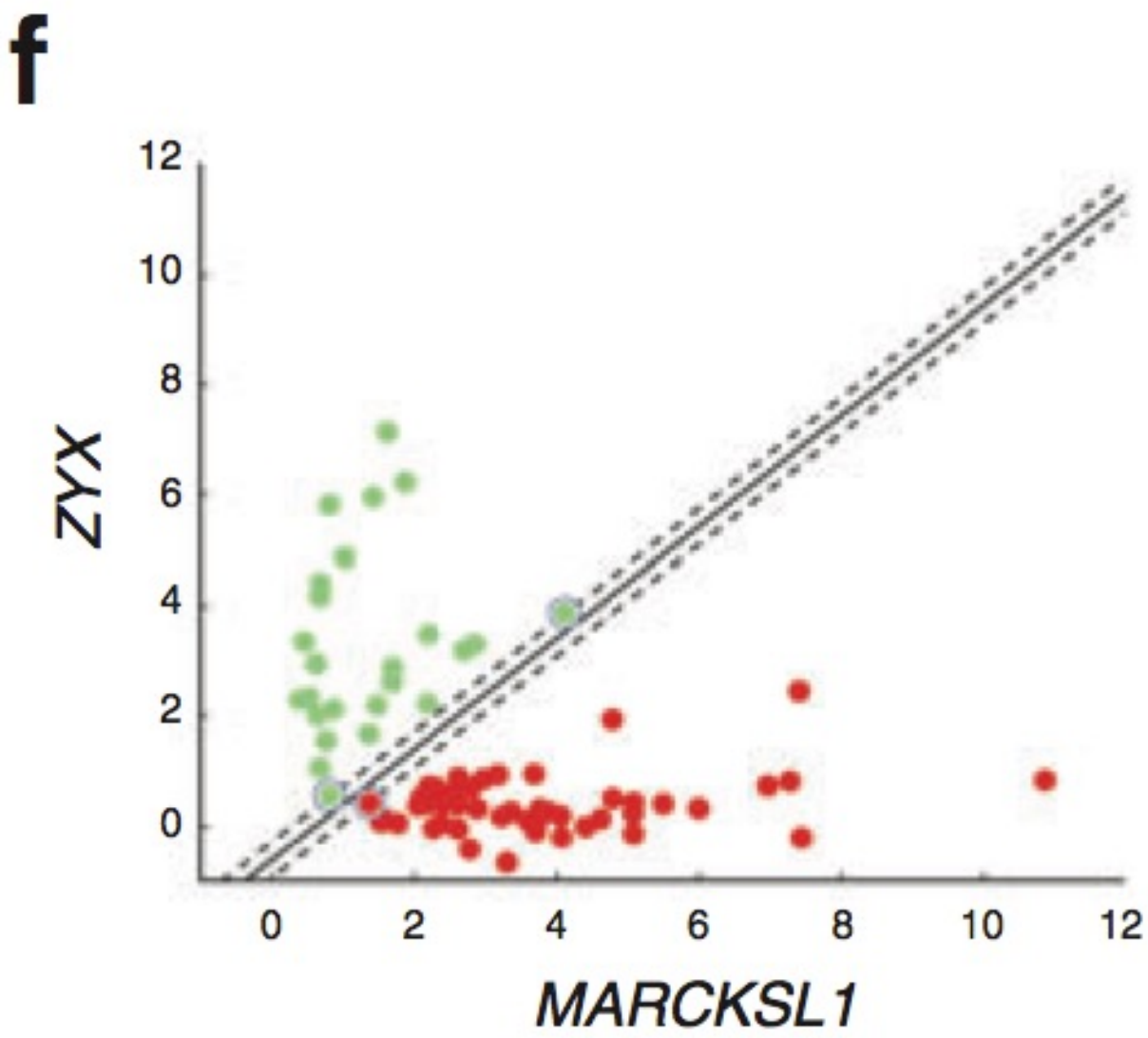
- In the case of more than two genes, a line generalizes to a plane or "hyperplane".
- For generality, we refer to them all as "hyperplane"

William S Nobel. What is a support vector machine? Nature Biotechnology. 2006



- Is there a “best” line?

William S Nobel. What is a support vector machine? Nature Biotechnology. 2006



- The maximum margin hyperplane

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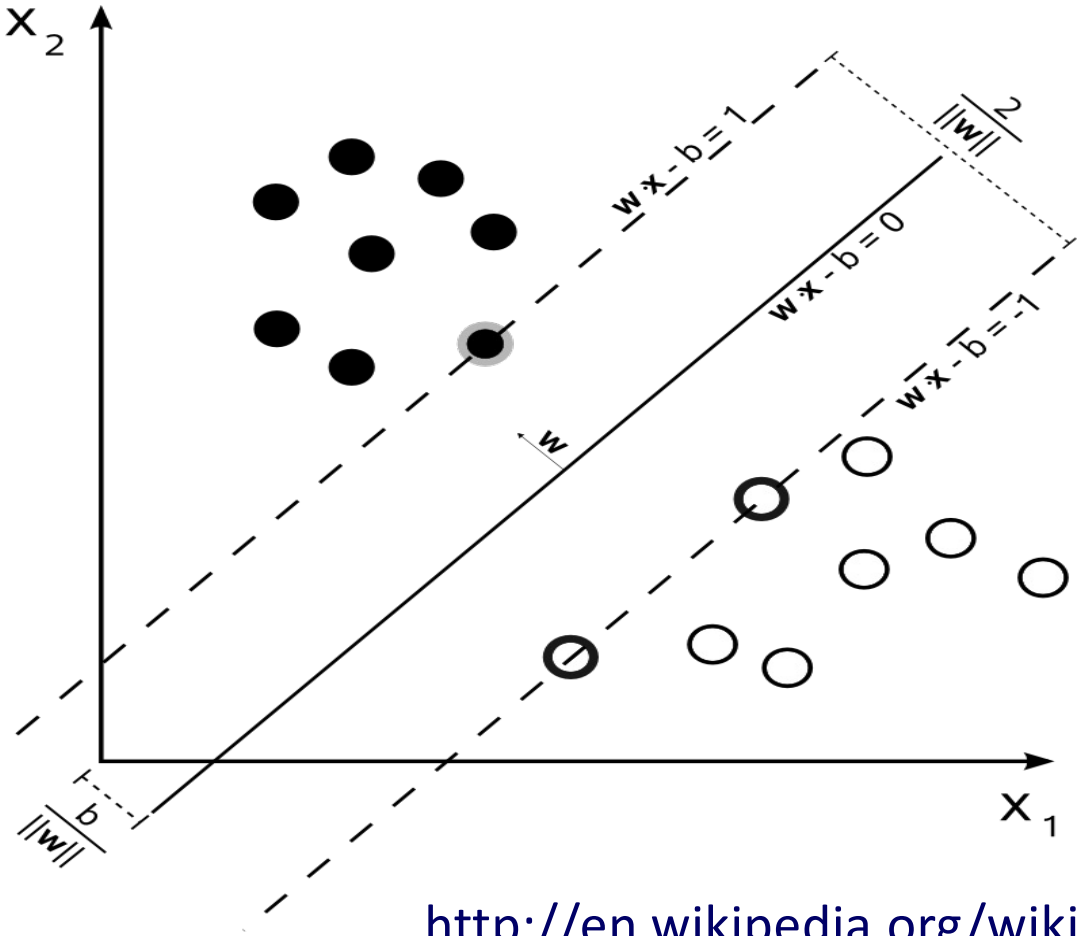
- Denote each data point as (x_i, y_i)
- x_i is a vector of the expression profiles
- y_i = -1 or 1, which labels the class
- A hyperplane can be represented as: w*x + b = 0
- The margin-width equals to: $2/\|w\|, \|w\| = \sqrt{w \bullet w}$



http://en.wikipedia.org/wiki/Support_vector_machine

- Find a hyperplane such that:
  - No data points fall between the lines $w \bullet x + b = -1$ and $w \bullet x + b = +1$
  - The margin 2/||w|| is maximized

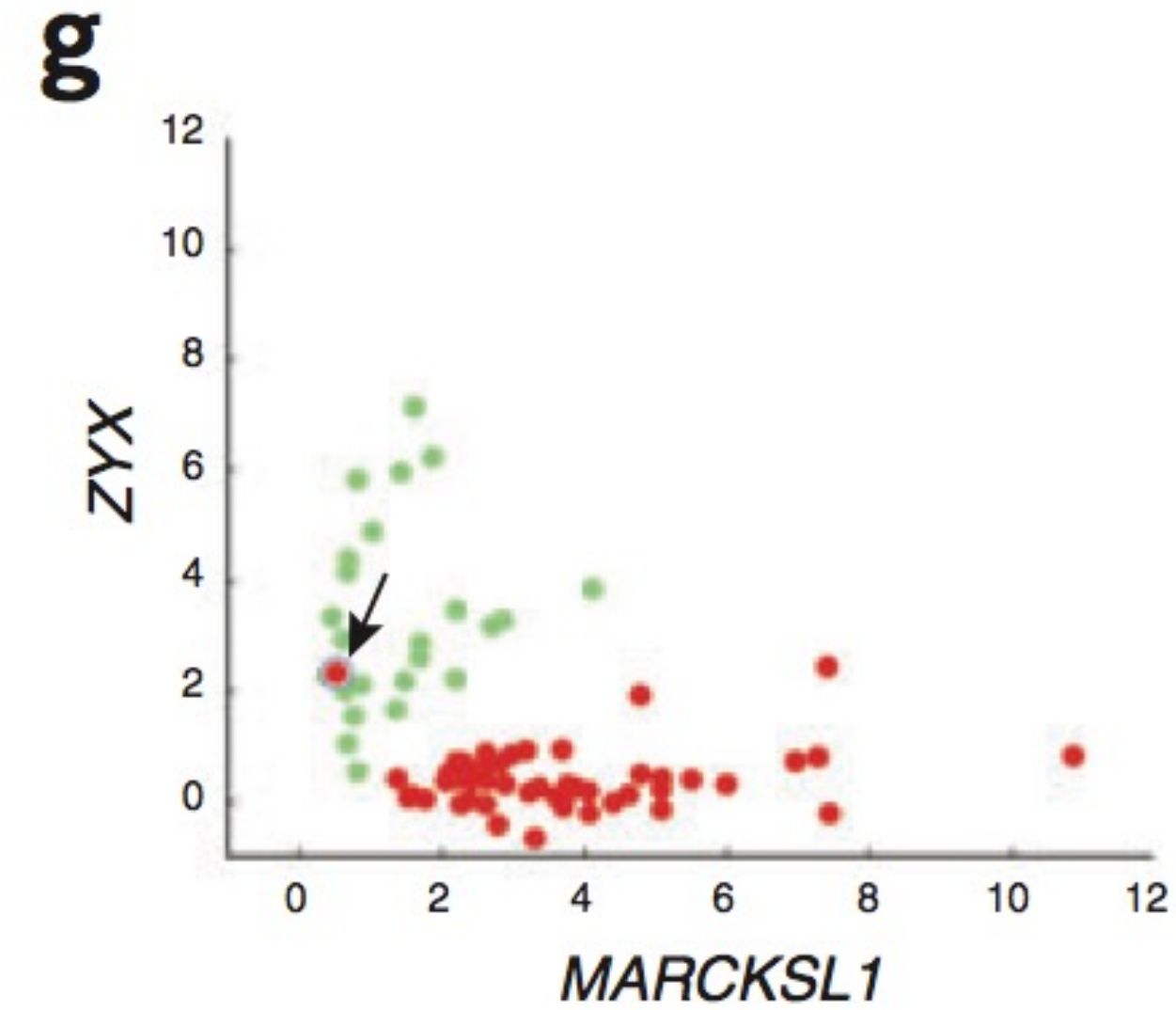
- Mathematically,
  - Minimize$_{w,b}$ ½*||w||^2, subject to:
  - for $y_i = 1$, $w \bullet x_i + b \geq 1$
  - for $y_i = -1$, $w \bullet x_i + b \leq -1$
  - Combining them, for any i, $y_i(w \bullet x_i + b) \geq 1$

- The solution expresses *w* as a linear combination of the x_i

- Assuming that the data points from two classes are always easily linearly separable. But that's not always the case

http://en.wikipedia.org/wiki/Support_vector_machine

- What if…



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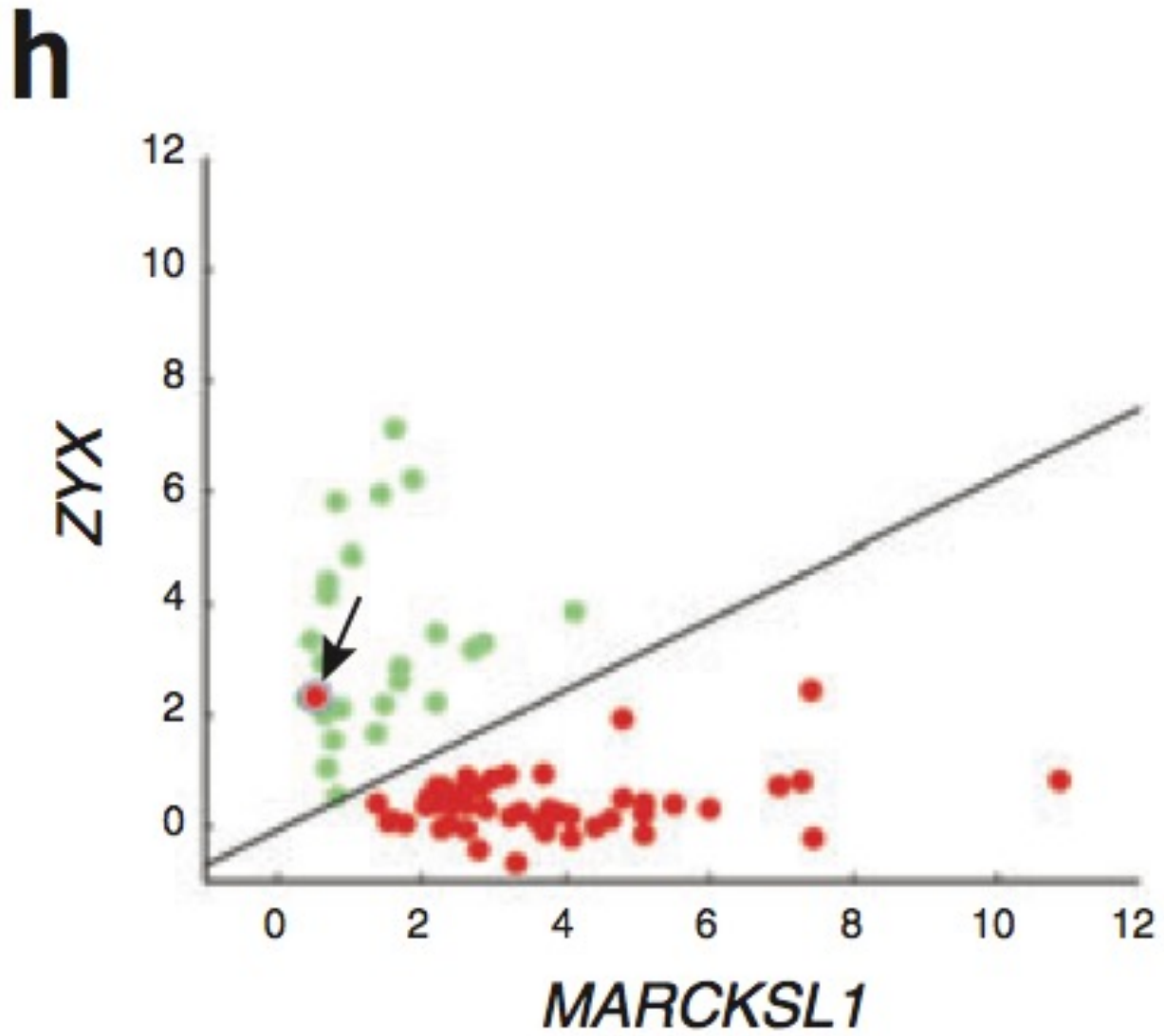
- Allow a few anomalous data points



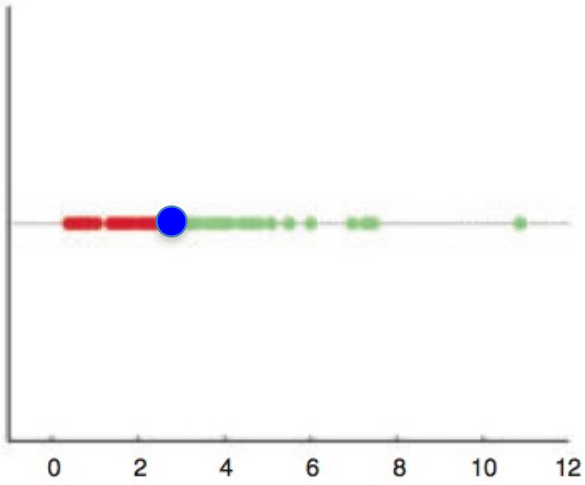
William S Nobel. What is a support vector machine? Nature Biotechnology. 2006

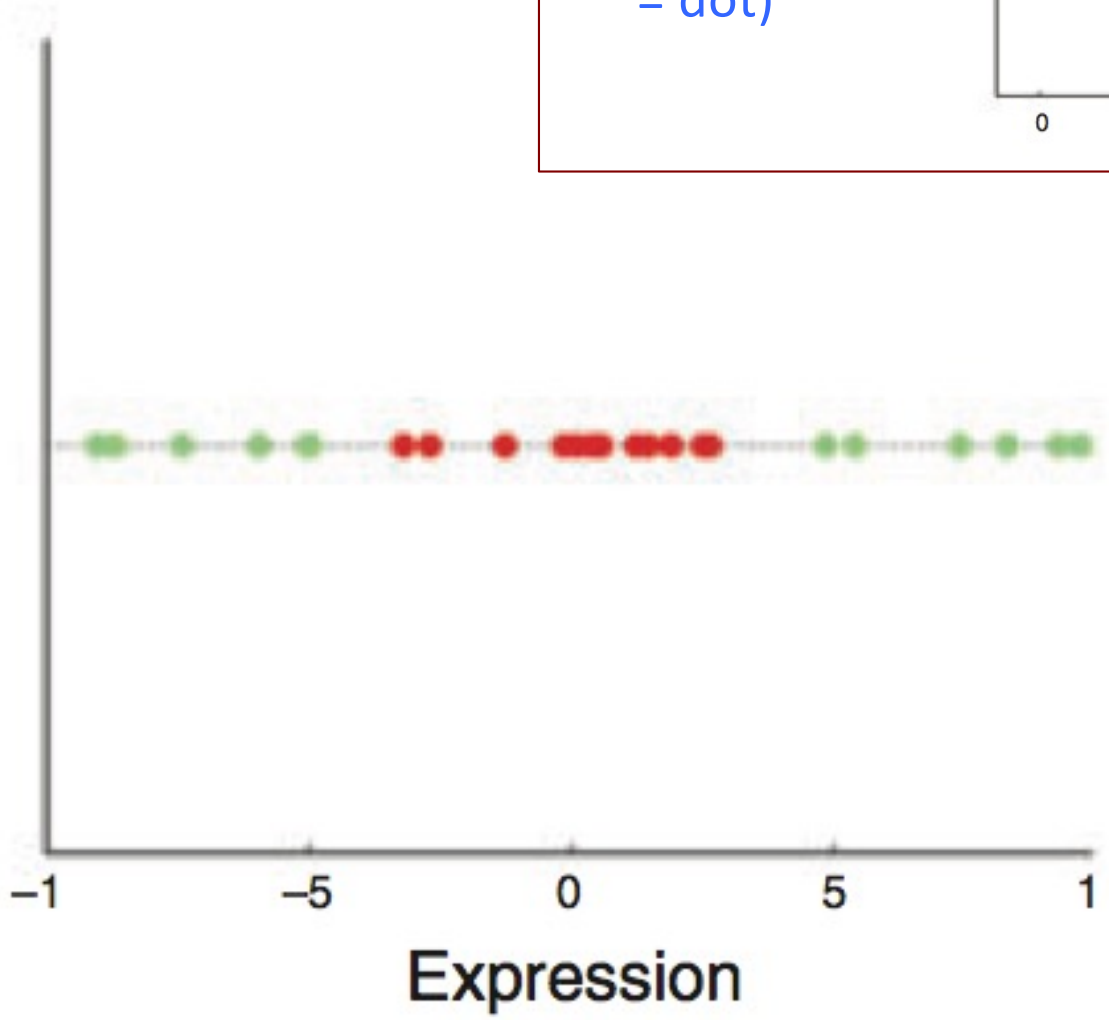
- The soft-margin SVM
  - $\underset{w,b,s}{\text{minimize}} \frac{1}{2} \| w \|^2 + C \sum_i s_i$
  - subject to, for any *i*, $y_i(w \bullet x_i + b) \geq 1 - s_i, s_i \geq 0$
  - S_i are the slack variables
  - *C* controls the number of tolerated misclassifications
    (It's effectively a regularization parameter on model complexity)
  - A small *C* would allow more misclassifications
  - A large *C* would discourage misclassifications
  - Note that even when the data points are linearly separable, one can still introduce the slack variables to pursue a larger separation margin

http://en.wikipedia.org/wiki/Support_vector_machine

- Are linear separating hyperplanes enough?

Yes
(by a 1D-hyperplane = dot)





NO

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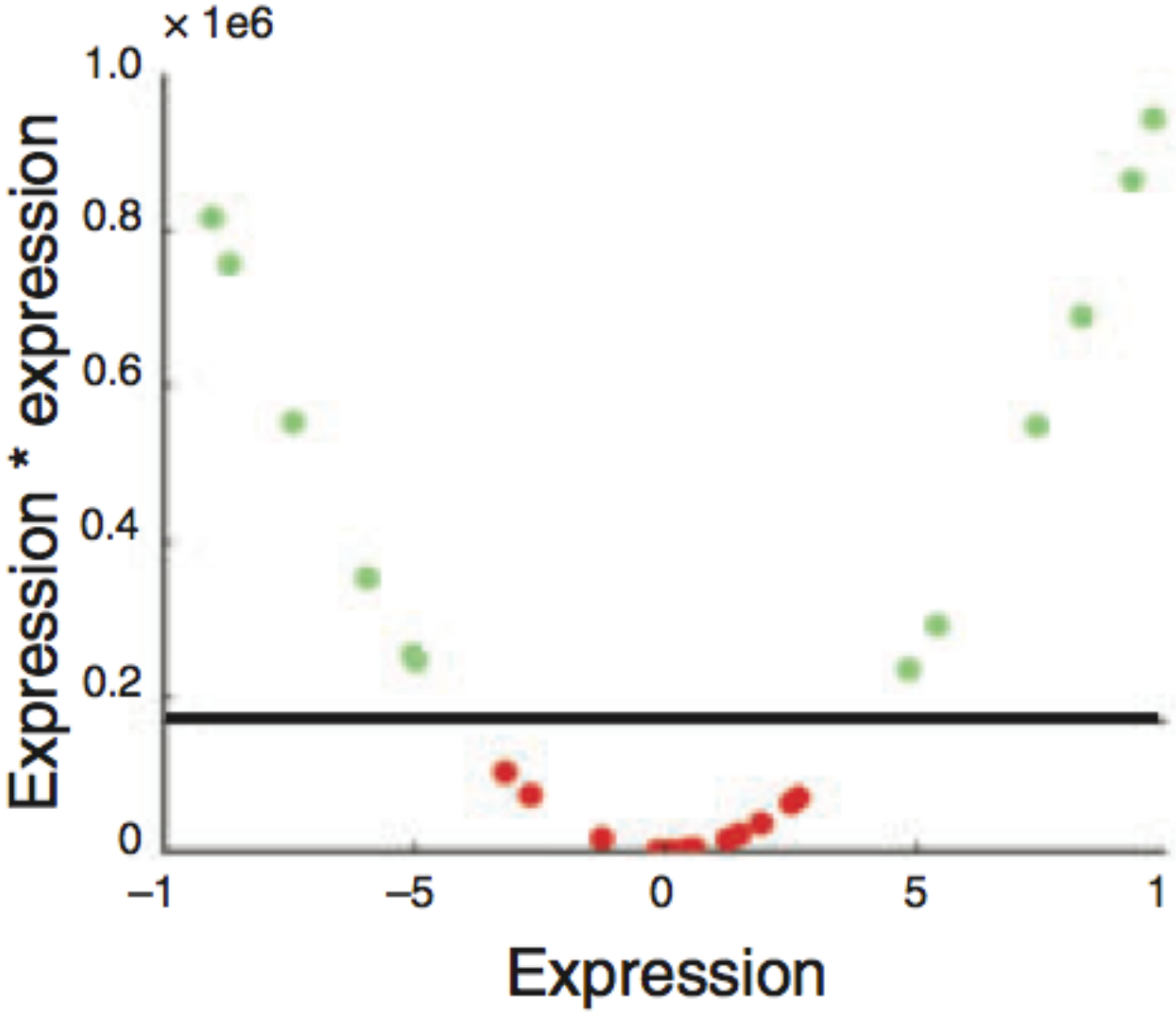
- Transform (x_i) into (x_i, x_i^2)



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Non-linear SVM

- In some cases (e.g. the above example), even soft-margin cannot solve the non-separable problem
- Generally speaking, we can apply some function to the original data points so that different classes become linearly separable (maybe with the help of soft-margin)
  - In the above example, the function is $f(x) = (x, x^2)$
- The most import trick in SVM: to allow for the transformation, we only need to define the "kernel function", $k(x_i, x_j) = f(x_i) \bullet f(x_j)$
  - e.g., a polynomial kernel used in above example

Solving SVM

– Formulation of SVM using Lagrangian multipliers

$$Minimize\ \frac{\|w\|^2}{2} + \sum_i \alpha_i (1 - y_i (w^T x_i + b))$$

– The dual formulation of SVM can be expressed as:

$$\underset{\alpha}{\text{Maximize}} \sum_i \alpha_i - \frac{1}{2} \sum_{i,j} y_i y_j \alpha_i \alpha_j x_i \bullet x_j \text{ , subject to}$$

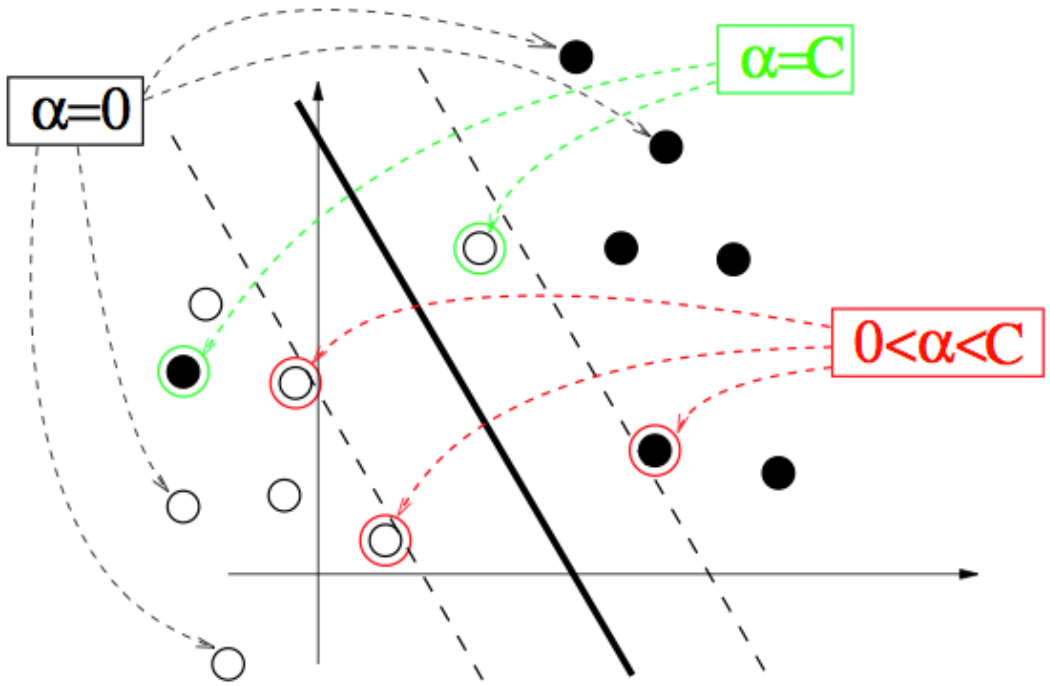
$$\sum_i y_i \alpha_i = 0, 0 \le \alpha_i \le C$$

no *w* and *b* now

– The "Kernel": $x_i \bullet x_j$ can be replaced by more sophisticated kernel functions:

$$k(x_i, x_j) = f(x_i) \bullet f(x_j)$$

Support vectors



- The x_i for which $\alpha_i > 0$ are called support vectors
- They fall between or right on the separating margins

from http://cbio.ensmp.fr/~jvert/talks/110401mines/mines.pdf

Tricks for solving SVM

- Finding optimal *w* & *b* can be replaced by finding optimal "Lagrange multipliers" α_i
  - One only optimizes using the product of $x_i * x_j$, now expressing the solution in terms of positive α_i for x_i that function as support vectors

- Non-linear SVM $x_i * x_j$ is replaced by $f(x_i) * f(x_j)$, so you don't need to know $f(x_i)$ itself only the product
  - *Kernel trick*: $f(x_i) * f(x_j)$ is just replaced by $k(x_i, x_j)$. That is, one only has to know the "distance" between x_i & x_j in the high-dimensional space -- not their actual representation

Kernel functions

- Polynomial kernel:
  - $k(x_i, x_j) = (x_i \bullet x_j + a)^d$
  - a = 1 (inhomogeneous) or 0 (homogenous)
  - *d* controls the degree of polynomial and henceforth the flexibility of the classifier
  - degenerates to linear kernel when a = 0 and d = 1
- Gaussian kernel:
  - $k(x_i, x_j) = (-1/\sigma \| x_i - x_j \|^2)$
  - σ controls the width of the Gaussian and plays a similar role as d in the polynomial kernels

Kernel functions in computational biology

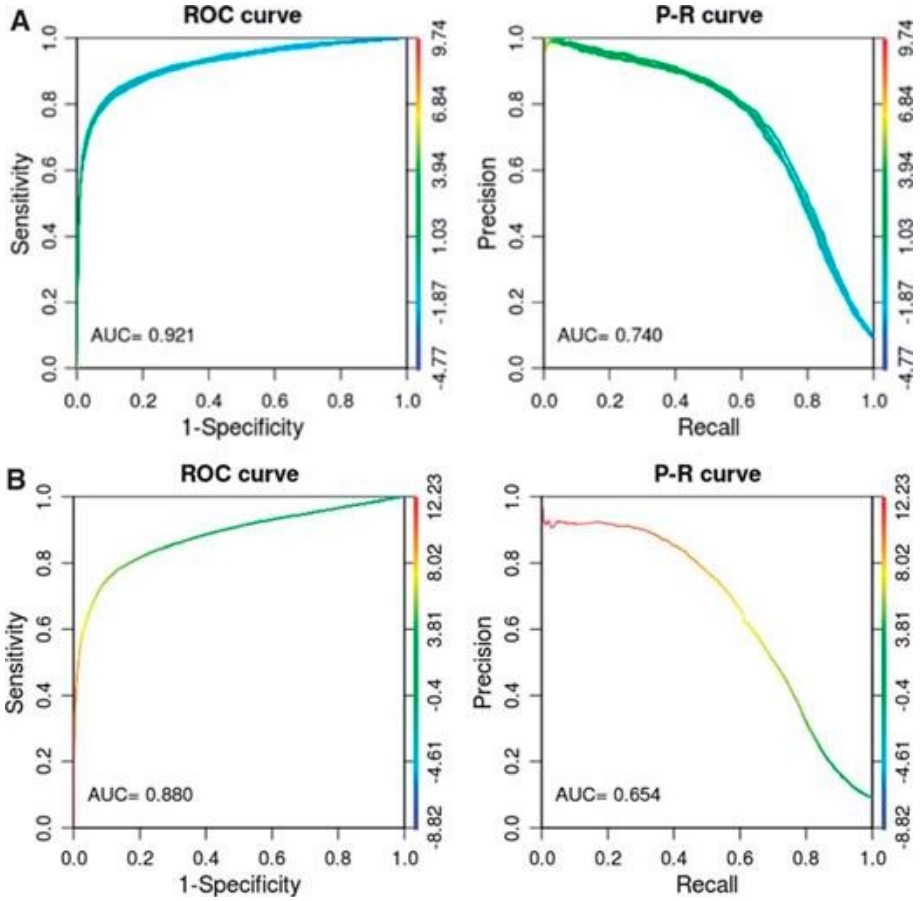
- ”Distance” even for non-vector biological data
  - Protein-protein interactions
  - DNA binding
  - Ben-Hur et al., Support Vector Machines and Kernels for Computational Biology, PLoS Comp. Bio., 2008
- For example, “Spectrum kernels” for sequences
  - k-spectrum of a sequence *x* is all possible k-length subsequence
  - Map the sequence to counts on k-spectrum *c*(*x*)
  - Spectrum kernel K_k(x,y)=<c(x), c(y)>
  - Leslie et al., PSB, 2002

kmer-SVM for predicting regulatory sequence features

- Fletez-Brant et al., NAR, 2013
- For example, ESRRB binding sites

kmer-SVM

PWM



C

6-mers	Revcomp	SVM Scores
Positive 6-mers		
AAGGTC	GACCTT	10.05
AGGTCA	TGACCT	8.47
ACCTTG	CAAGGT	5.33
AGGTCG	CGACCT	5.17
GGTCAA	TTGACC	4.01
Negative 6-mers		
GCAATA	TATTGC	-2.05
TGACCA	TGGTCA	-3.33
AAGGTA	TACCTT	-4.23
AGACCT	AGGTCT	-4.55
AGGTCC	GGACCT	-4.98

D

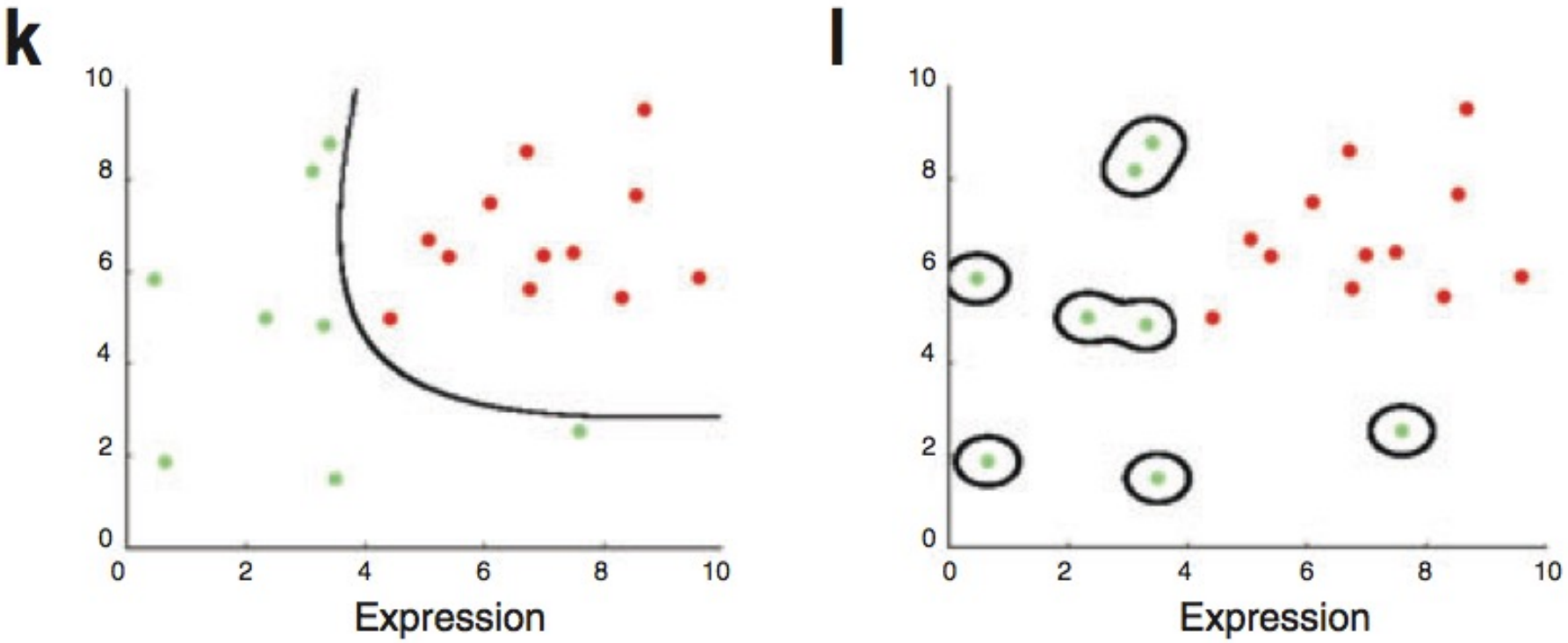
Esrrb

Avoid over-fitting by kernel functions

- High-degree kernels always fit the training data well, but at increased risks of over-fitting, i.e. the classifier will not generalize to new data points

- One needs to find a balance between classification accuracy on the training data and regularity of the kernel (not allowing the kernel to be too flexible)

A low-degree kernel (left) and an over-fitting high-degree kernel (right)



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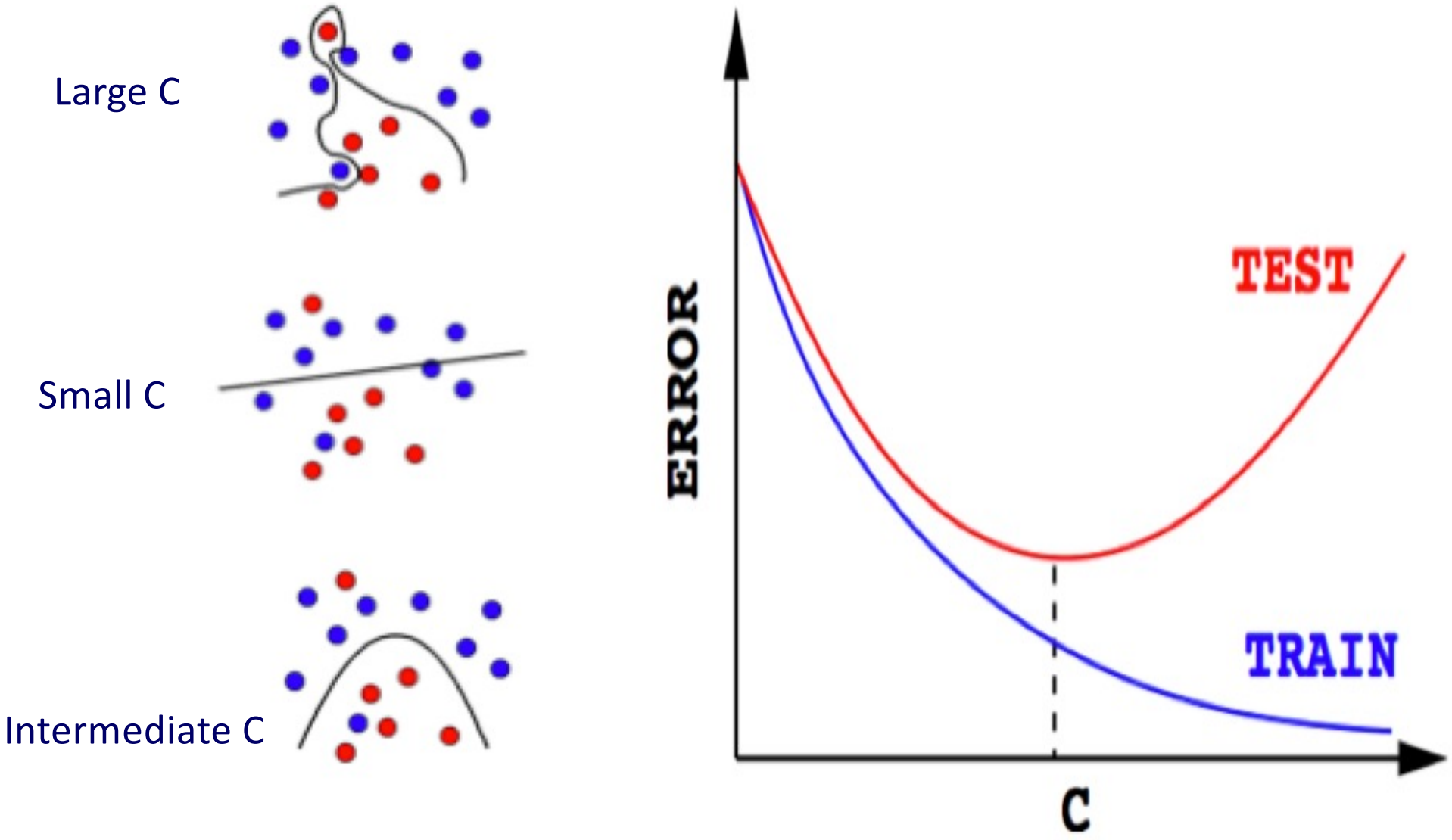
The parameter C has a similar role

- Large C will make few classification errors on the training data
- But this may not generalize to the testing data
- Small C pursues a large separating margin at the expenses of some classification errors on the training data.
- The accuracy more likely to generalize to testing data

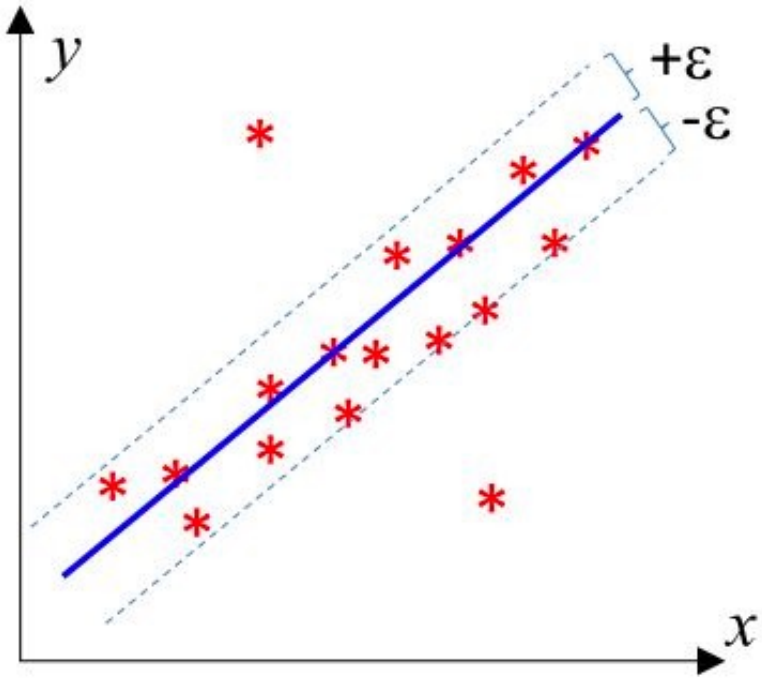


http://cbio.ensmp.fr/~jvert/talks/110401mines/mines.pdf

ε-Support vector regression (ε-SVR)

Given training data: $\vec{x}_1, \vec{x}_2, \ldots, \vec{x}_N \in R^n$

$y_1, y_2, \ldots, y_N \in R$



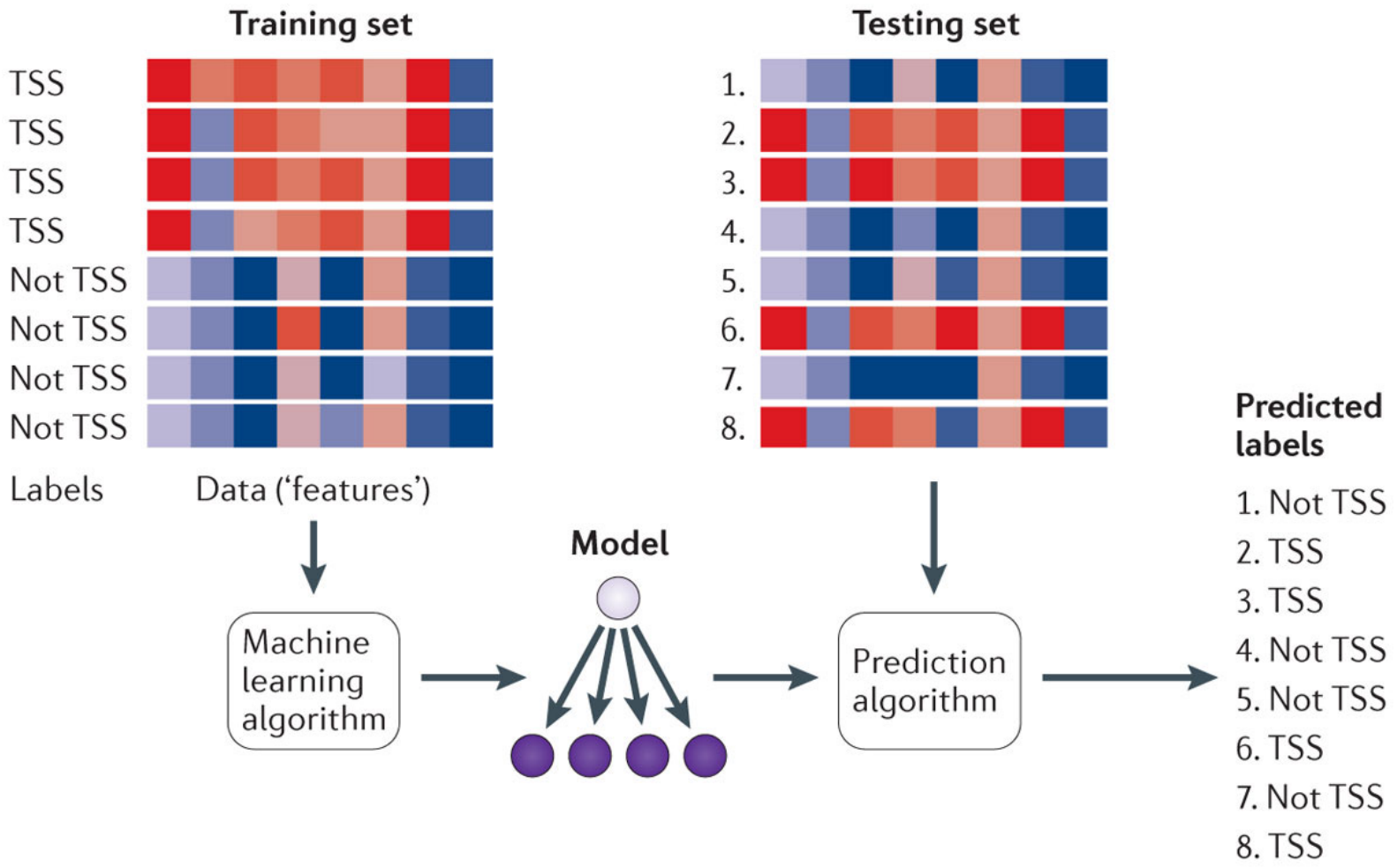
Main idea:

Find a function $f(\vec{x}) = \vec{w} \cdot \vec{x} + b$ that approximates $y_1, \ldots, y_N$:

- it has at most ε derivation from the true values y_i
- it is as "flat" as possible (to avoid overfitting)

E.g., build a model to predict survival of cancer patients that can admit a one month error (= ε).

Training and Testing



Libbrecht, M., Noble, W. Machine learning applications in genetics and genomics. *Nat Rev Genet* **16,** 321–332 (2015). https://doi.org/10.1038/nrg3920

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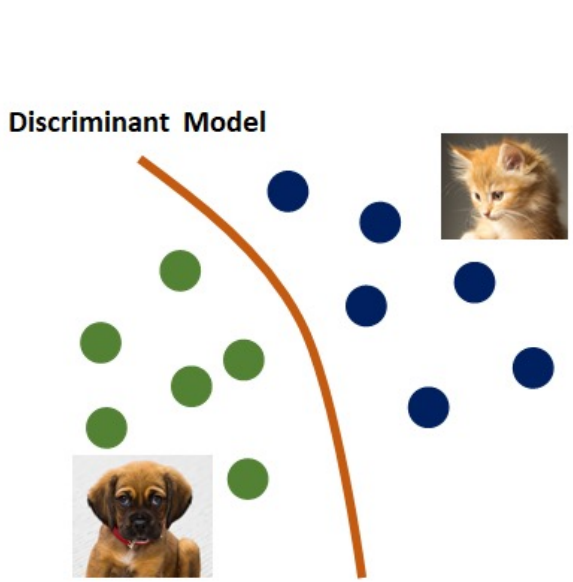
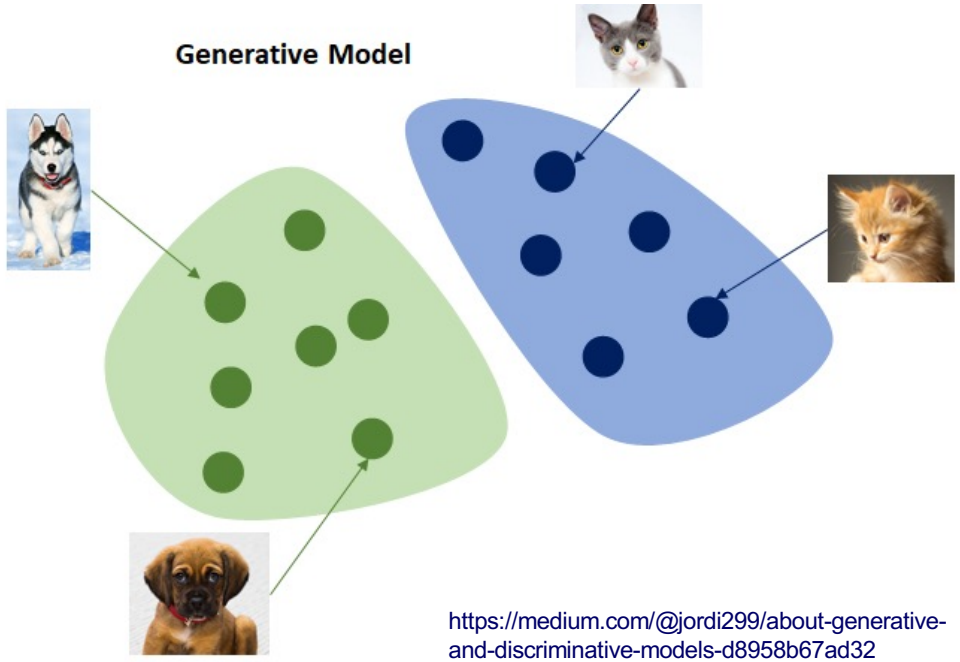
Generative vs. Discriminative models

- Generative approaches model the joint probability p(x,y) for generating data

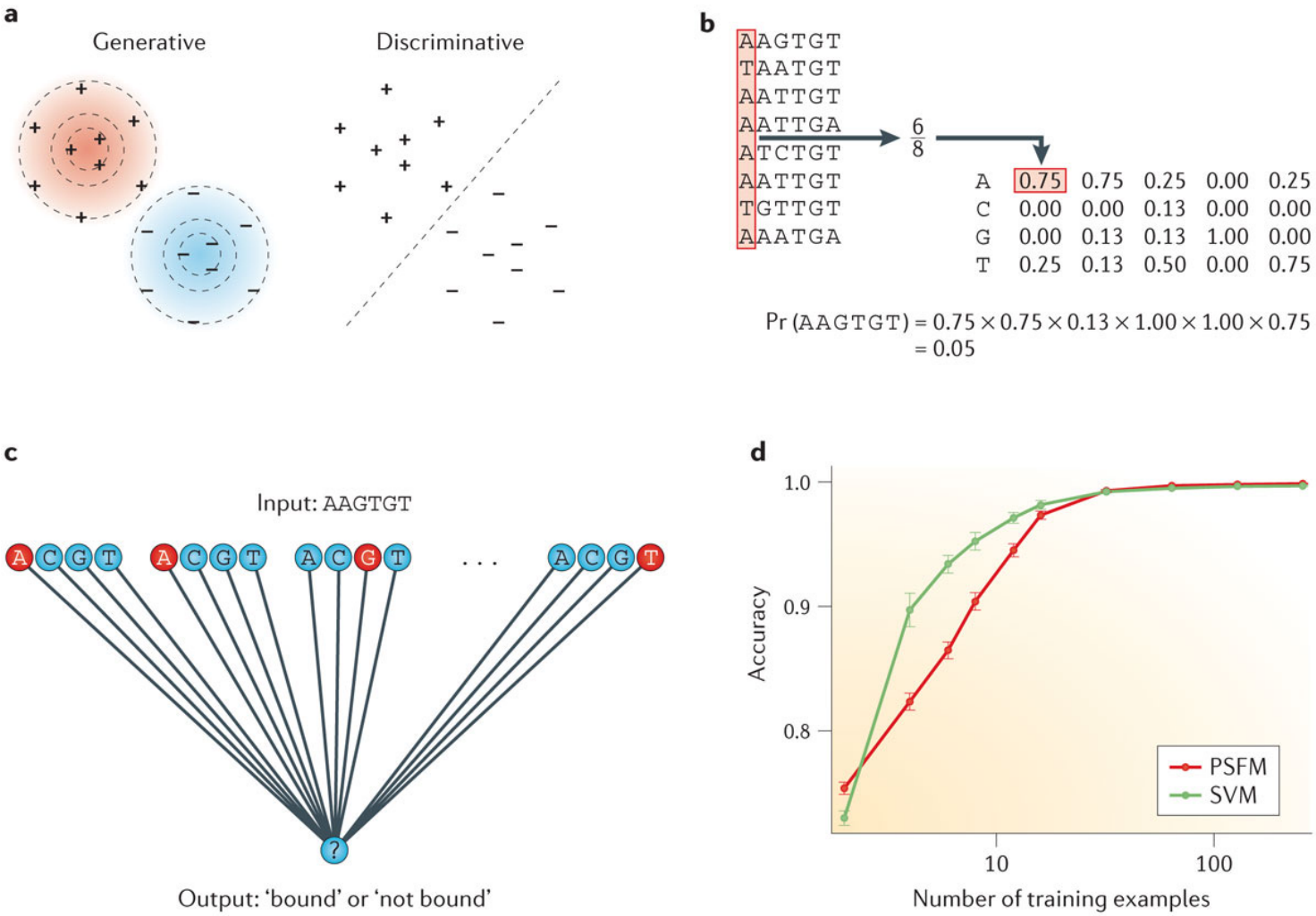
- Discriminative approaches directly model p(y|x) for classification





https://medium.com/@jordi299/about-generative-and-discriminative-models-d8958b67ad32

Predicting TF binding via Generative vs. Discriminative models



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Workshop introducing machine learning to biologists

- ML4BIO workshop from Gitter Lab
- https://gitter-lab.github.io/ml-bio-workshop/