Classifiers!!!

BCH394P/364C Systems Biology / Bioinformatics

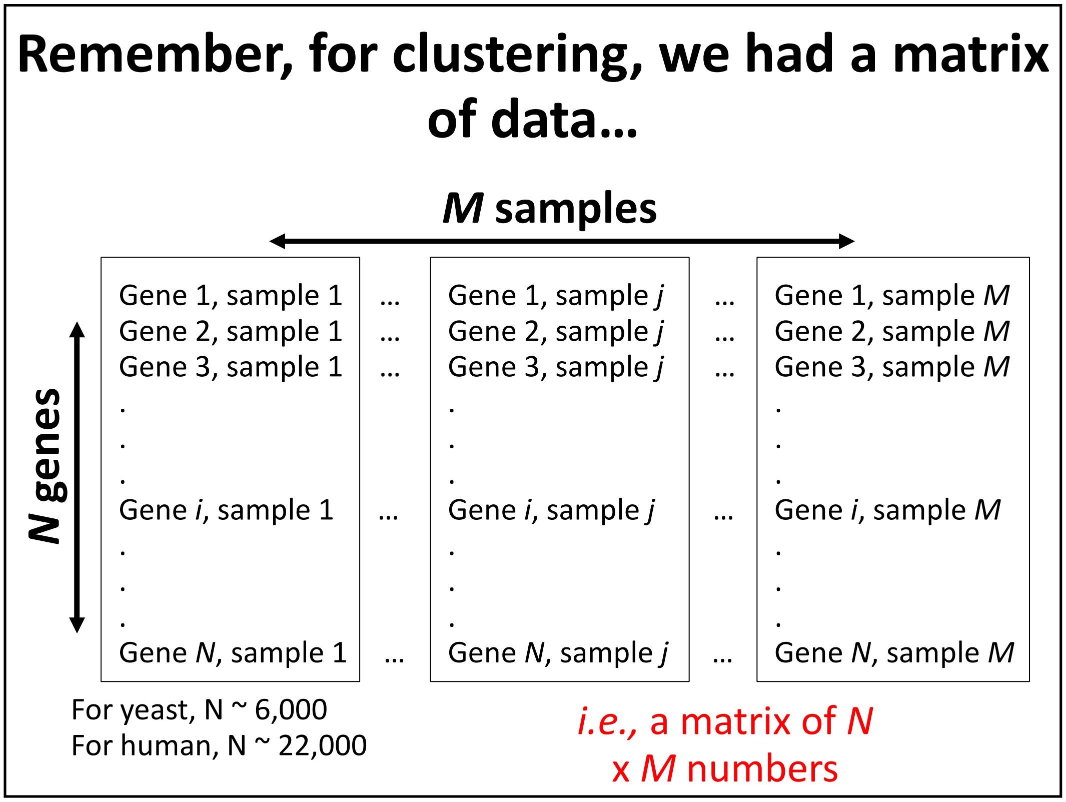
Edward Marcotte, Univ of Texas at Austin

Clustering = task of grouping a set of objects in such a way that objects in the same group (a **cluster**) are more similar (in some sense) to each other than to those in other groups (clusters).

VS.

Classification = task of categorizing a new observation, on the basis of a training set of data with observations (or instances) whose categories are known

Adapted from Wikipedia



Remember, for clustering, we had a matrix of data…

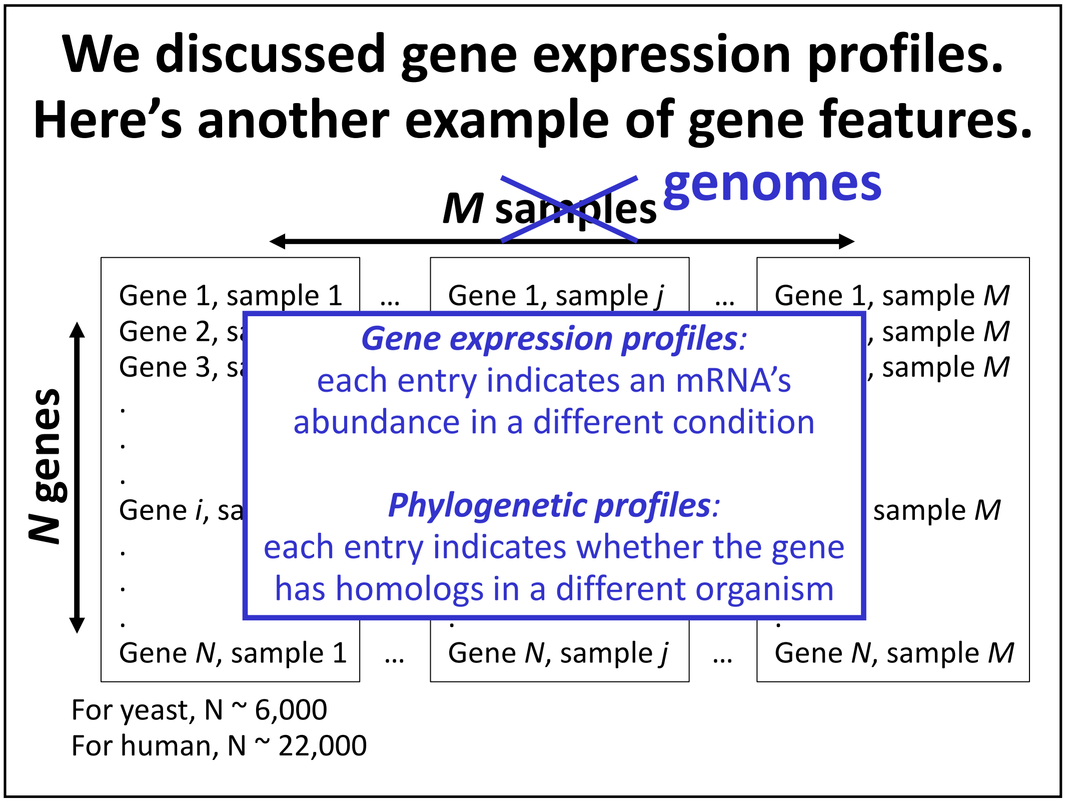
***M* samples**

***N* genes**

Gene 1, sample 1	…	Gene 1, sample *j*	…	Gene 1, sample *M*
Gene 2, sample 1	…	Gene 2, sample *j*	…	Gene 2, sample *M*
Gene 3, sample 1	…	Gene 3, sample *j*	…	Gene 3, sample *M*
.		.		.
.		.		.
.		.		.
Gene *i*, sample 1	…	Gene *i*, sample *j*	…	Gene *i*, sample *M*
.		.		.
.		.		.
.		.		.
Gene *N*, sample 1	…	Gene *N*, sample *j*	…	Gene *N*, sample *M*

For yeast, N ~ 6,000
For human, N ~ 22,000

i.e., a matrix of *N* x *M* numbers



We discussed gene expression profiles. Here's another example of gene features.

***M* ~~samples~~ genomes**

***N* genes**

Gene 1, sample 1	…	Gene 1, sample *j*	…	Gene 1, sample *M*
Gene 2, sa				, sample *M*
Gene 3, sa				, sample *M*
.				
.				
.				
Gene *i*, sa				sample *M*
.				
.				
.		.		.
Gene *N*, sample 1	…	Gene *N*, sample *j*	…	Gene *N*, sample *M*

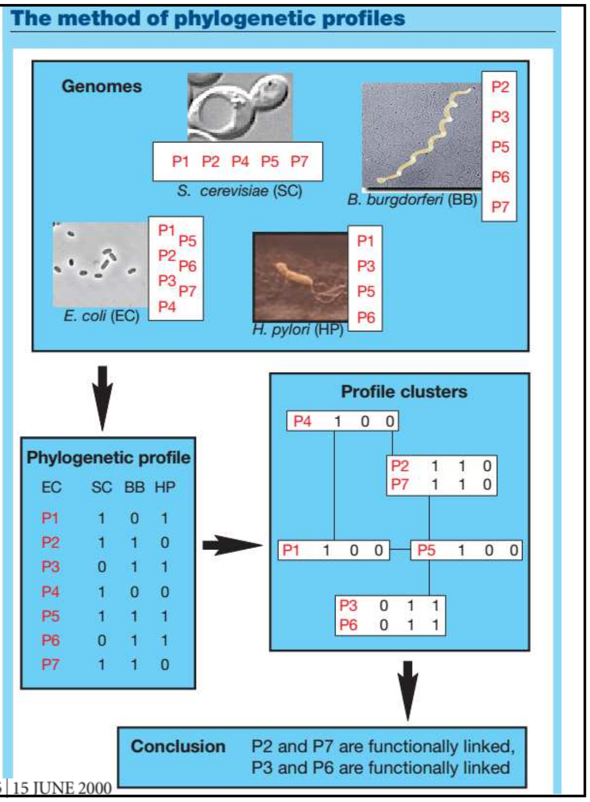
***Gene expression profiles*:**
each entry indicates an mRNA's abundance in a different condition

***Phylogenetic profiles*:**
each entry indicates whether the gene has homologs in a different organism

For yeast, N ~ 6,000
For human, N ~ 22,000

This is useful because biological systems tend to be modular and often inherited intact across evolution.

(e.g. you tend to have a flagellum or not)



Phylogenetic profile

EC	SC	BB	HP
P1	1	0	1
P2	1	1	0
P3	0	1	1
P4	1	0	0
P5	1	1	1
P6	0	1	1
P7	1	1	0

Conclusion P2 and P7 are functionally linked, P3 and P6 are functionally linked

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Many such features are possible…

M samples

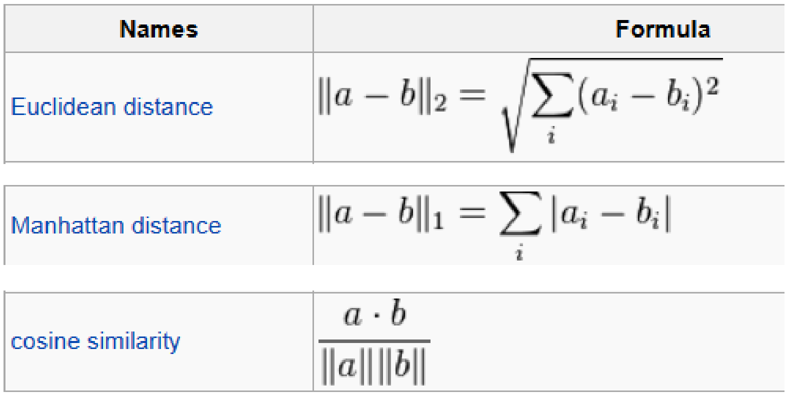
N genes

Gene 1, sample 1	…	Gene 1, sample *j*	…	Gene 1, sample *M*
Gene 2, sample 1	…	Gene 2, sample *j*	…	Gene 2, sample *M*
Gene 3, sample 1	…	Gene 3, sample *j*	…	Gene 3, sample *M*
.		.		.
.		.		.
.		.		.
Gene *i*, sample 1	…	Gene *i*, sample *j*	…	Gene *i*, sample *M*
.		.		.
.		.		.
.		.		.
Gene *N*, sample 1	…	Gene *N*, sample *j*	…	Gene *N*, sample *M*

For yeast, N ~ 6,000
For human, N ~ 22,000

i.e., a matrix of *N* x *M* numbers

We also needed a measure of the similarity between feature vectors. Here are a few (of many) common distance measures used in clustering.



Names	Formula
Euclidean distance	$\|a-b\|_2 = \sqrt{\sum_i (a_i - b_i)^2}$
Manhattan distance	$\|a-b\|_1 = \sum_i \|a_i - b_i\|$
cosine similarity	$\frac{a \cdot b}{\|a\|\|b\|}$

Wikipedia

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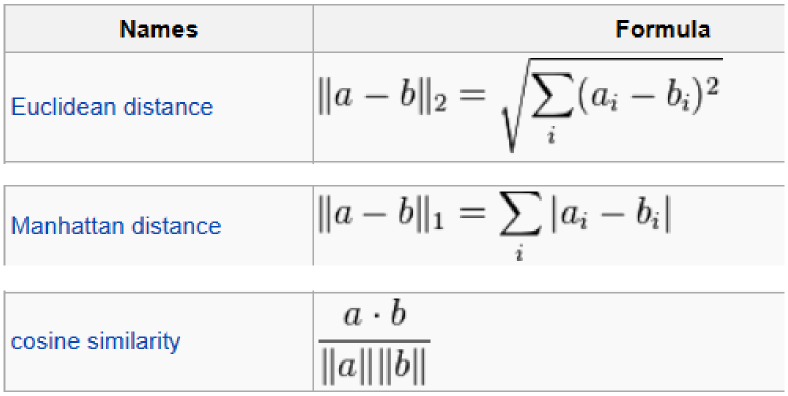

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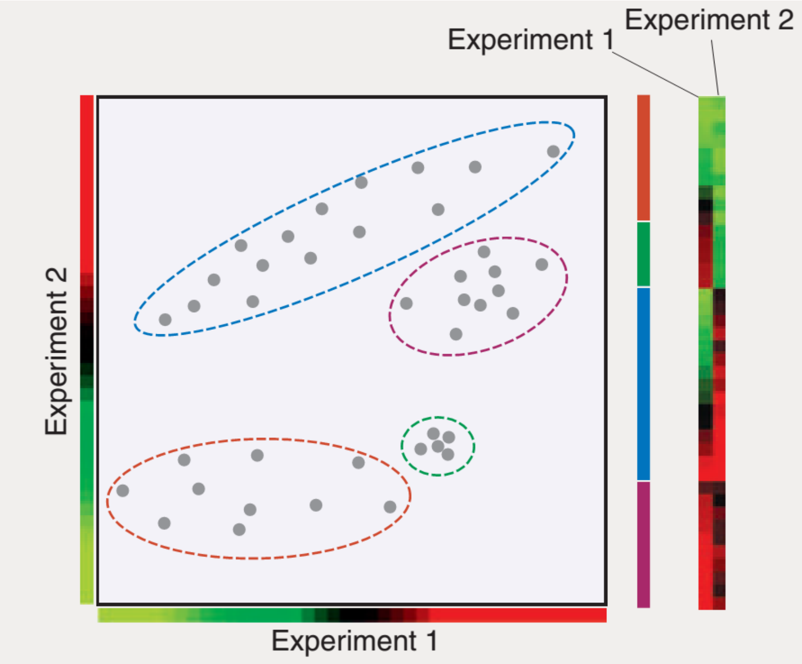
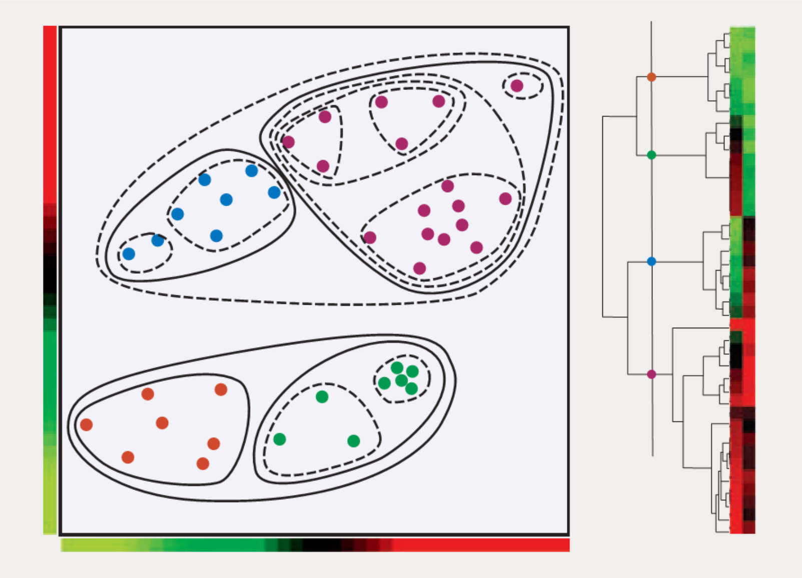
Wikipedia

Clustering refresher: 2-D example



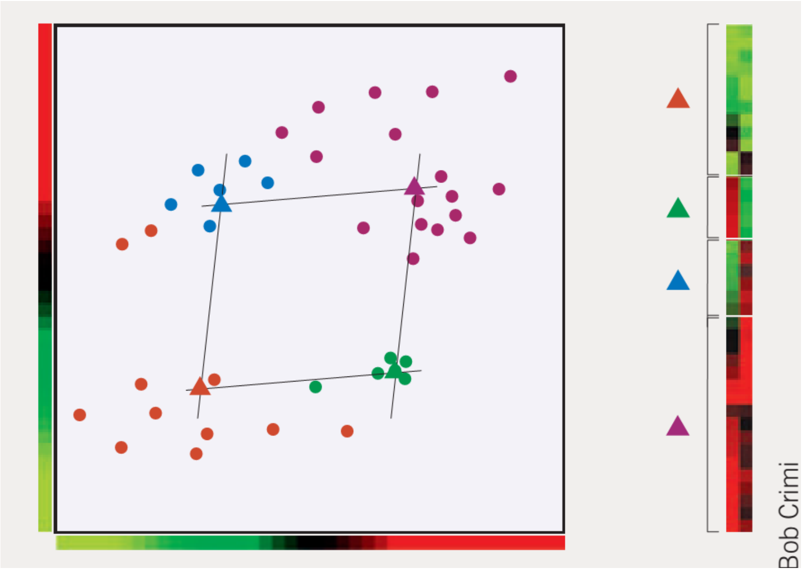
Nature Biotech 23(12):1499-1501 (2005)

Clustering refresher: hierarchical



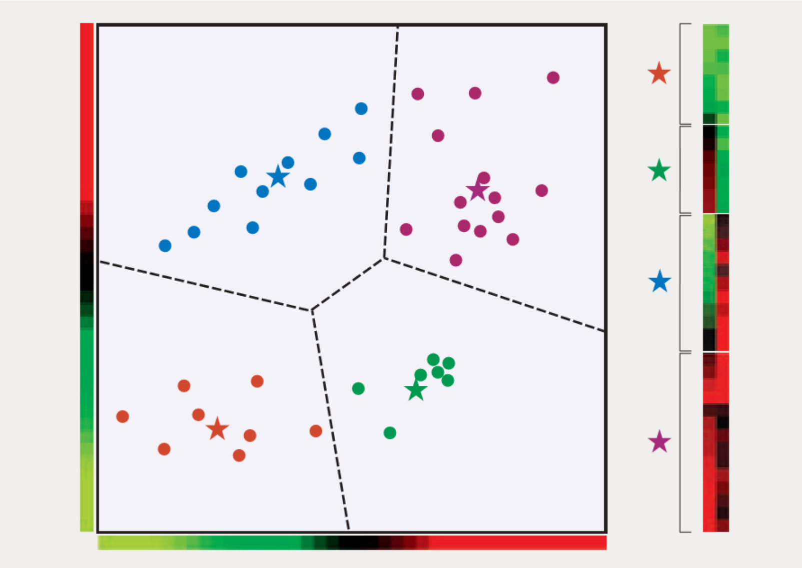
Nature Biotech 23(12):1499-1501 (2005)

Clustering refresher: SOM



Nature Biotech 23(12):1499-1501 (2005)

Clustering refresher: *k*-means



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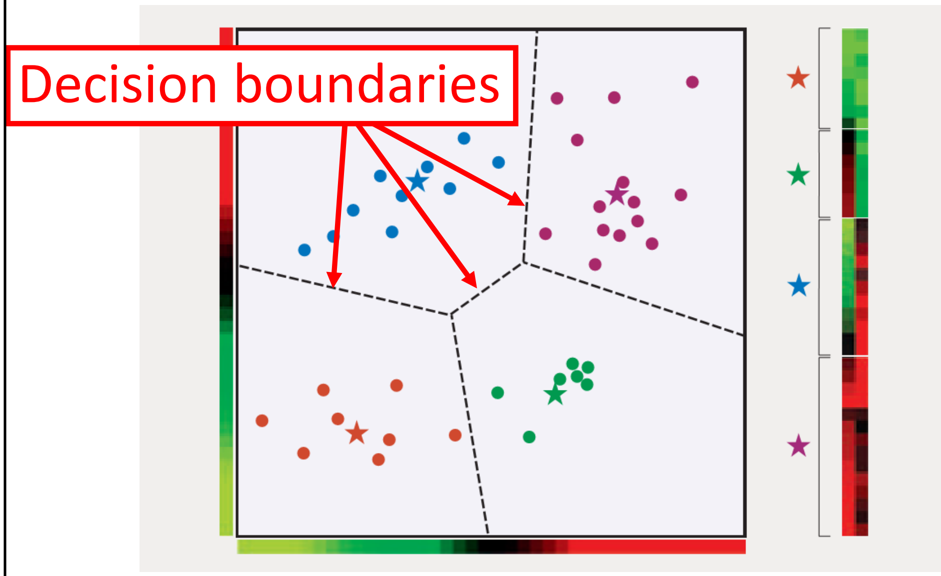
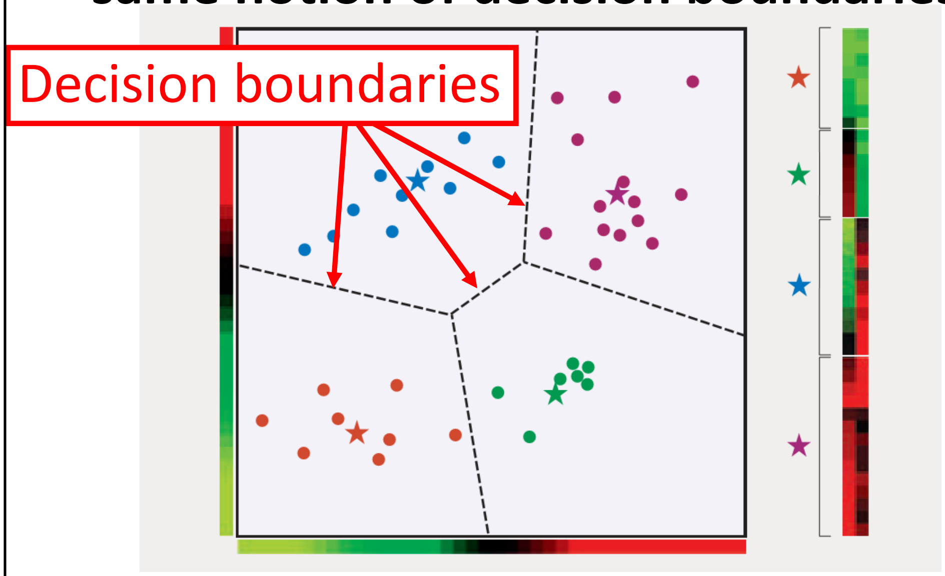
Clustering refresher: *k*-means



Decision boundaries

Nature Biotech 23(12):1499-1501 (2005)

One of the simplest classifiers uses the same notion of decision boundaries.



Decision boundaries

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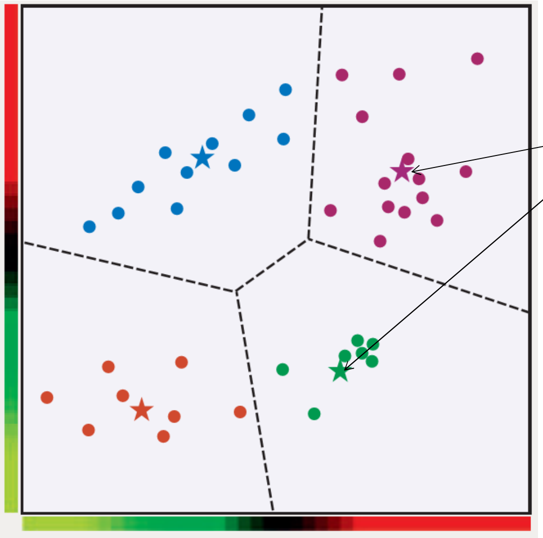


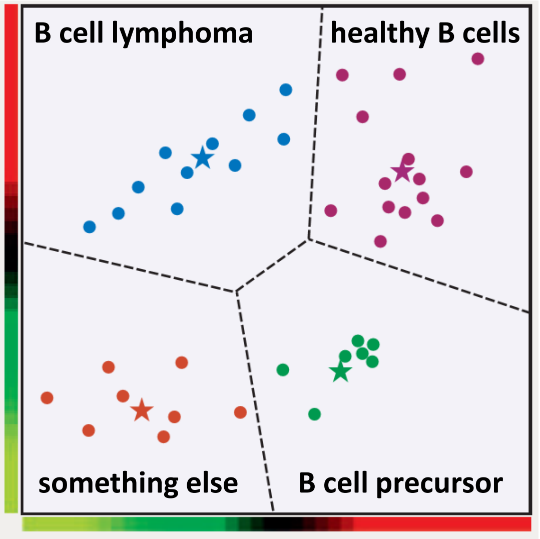
Rather than first clustering, calculate the centroid (mean) of objects with each label.

New observations are classified as belonging to the group whose mean is nearest.

="minimum distance classifier"

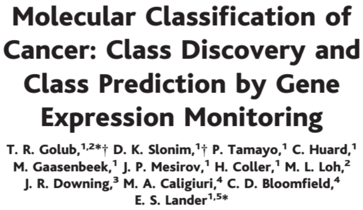
Nature Biotech 23(12):1499-1501 (2005)

One of the simplest classifiers uses this notion of decision boundaries.



For example….

Nature Biotech 23(12):1499-1501 (2005)



Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring

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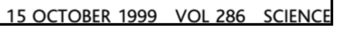
Let’s look at a specific historic example:

“Enzyme-based histochemical analyses were introduced in the 1960s to demonstrate that **some leukemias were periodic acid-Schiff positive, whereas others were myeloperoxidase positive…**

This provided the first basis for classification of acute leukemias into those arising
from <u>lymphoid</u> precursors (acute lymphoblastic leukemia, ALL), or from <u>myeloid</u> precursors (acute myeloid leukemia, AML).”



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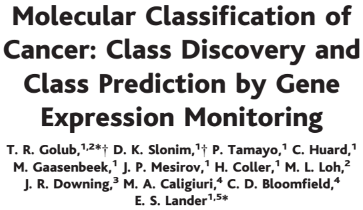

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Let’s look at a specific historic example:

“Distinguishing ALL from AML is critical for successful treatment…

chemotherapy regimens for ALL generally contain corticosteroids, vincristine, methotrexate, and L-asparaginase, whereas

most AML regimens rely on a backbone of daunorubicin and cytarabine (8).

Although remissions can be achieved using ALL therapy for AML (and vice versa), **<u>cure rates are markedly diminished</u>**, and unwarranted toxicities are encountered.”

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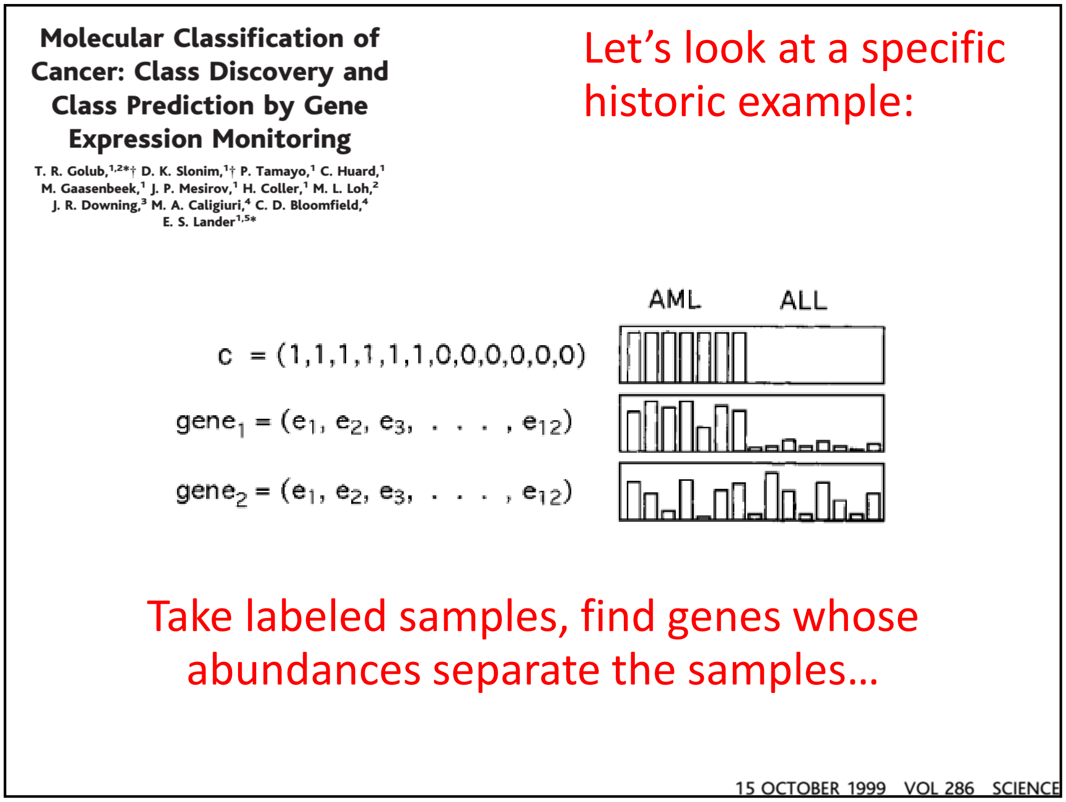

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Let's look at a specific historic example:

AML
ALL
$c = (1,1,1,1,1,1,0,0,0,0,0,0)$
$\mathrm{gene}_1 = (e_1, e_2, e_3, \ldots, e_{12})$
$\mathrm{gene}_2 = (e_1, e_2, e_3, \ldots, e_{12})$

Take labeled samples, find genes whose abundances separate the samples…

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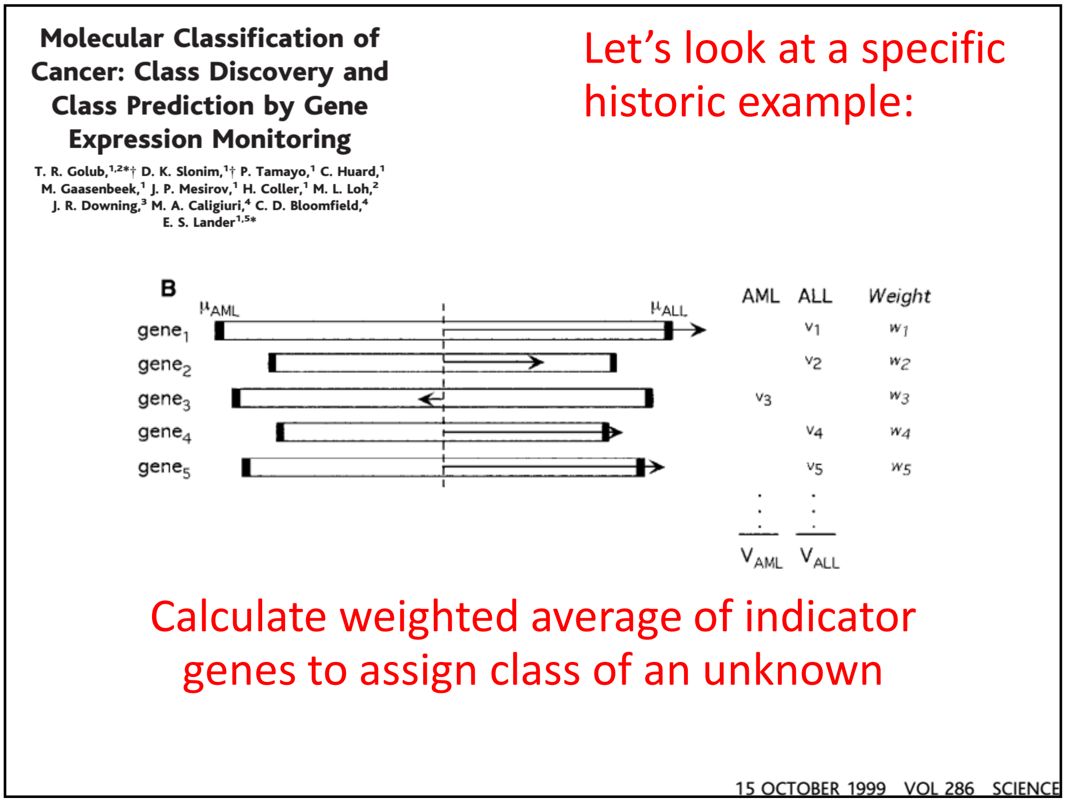

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Let's look at a specific historic example:

Calculate weighted average of indicator genes to assign class of an unknown

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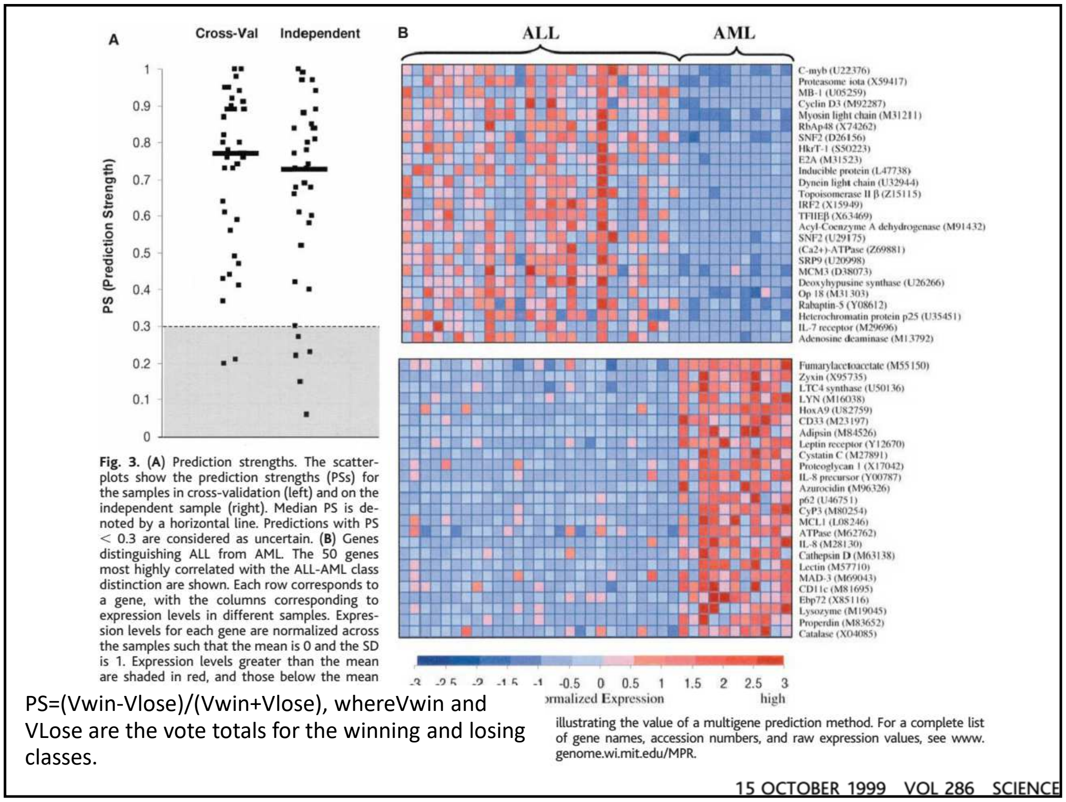

Fig. 3. (**A**) Prediction strengths. The scatterplots show the prediction strengths (PSs) for the samples in cross-validation (left) and on the independent sample (right). Median PS is denoted by a horizontal line. Predictions with PS < 0.3 are considered as uncertain. (**B**) Genes distinguishing ALL from AML. The 50 genes most highly correlated with the ALL-AML class distinction are shown. Each row corresponds to a gene, with the columns corresponding to expression levels in different samples. Expression levels for each gene are normalized across the samples such that the mean is 0 and the SD is 1. Expression levels greater than the mean are shaded in red, and those below the mean

PS=(Vwin-Vlose)/(Vwin+Vlose), whereVwin and VLose are the vote totals for the winning and losing classes.

illustrating the value of a multigene prediction method. For a complete list of gene names, accession numbers, and raw expression values, see www.genome.wi.mit.edu/MPR.

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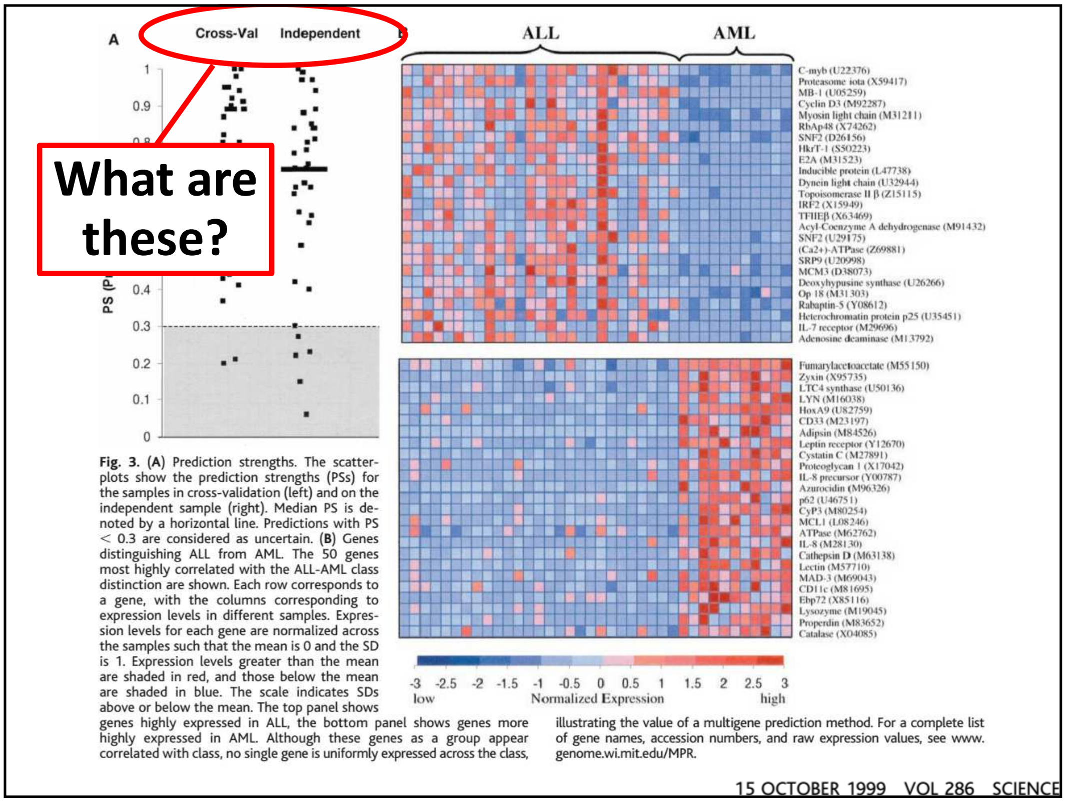
What are these?

Fig. 3. (**A**) Prediction strengths. The scatterplots show the prediction strengths (PSs) for the samples in cross-validation (left) and on the independent sample (right). Median PS is denoted by a horizontal line. Predictions with PS < 0.3 are considered as uncertain. (**B**) Genes distinguishing ALL from AML. The 50 genes most highly correlated with the ALL-AML class distinction are shown. Each row corresponds to a gene, with the columns corresponding to expression levels in different samples. Expression levels for each gene are normalized across the samples such that the mean is 0 and the SD is 1. Expression levels greater than the mean are shaded in red, and those below the mean are shaded in blue. The scale indicates SDs above or below the mean. The top panel shows genes highly expressed in ALL, the bottom panel shows genes more highly expressed in AML. Although these genes as a group appear correlated with class, no single gene is uniformly expressed across the class, illustrating the value of a multigene prediction method. For a complete list of gene names, accession numbers, and raw expression values, see www.genome.wi.mit.edu/MPR.

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Cross-validation

Withhold a sample, build a predictor based only on the remaining samples, and predict the class of the withheld sample.

Repeat this process for each sample, then calculate the cumulative or average error rate.

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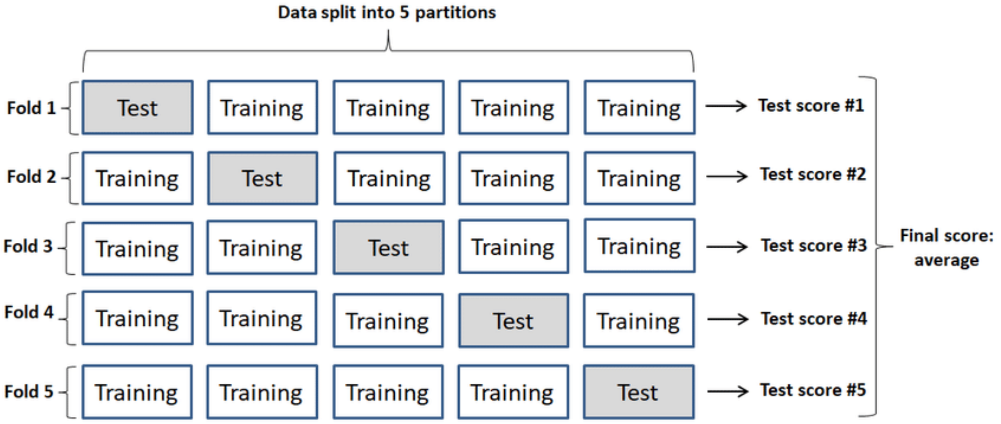
X-fold cross-validation
e.g. here, 5-fold:



Data split into 5 partitions

Fold 1	Test	Training	Training	Training	Training	Test score #1
Fold 2	Training	Test	Training	Training	Training	Test score #2
Fold 3	Training	Training	Test	Training	Training	Test score #3
Fold 4	Training	Training	Training	Test	Training	Test score #4
Fold 5	Training	Training	Training	Training	Test	Test score #5

Final score: average

Image CC-BY, from DOI:10.3390/app9214500

Independent data

Withhold an entire dataset, build a predictor based only on the remaining samples (the training data).

Test the trained classifier on the independent test data to give a fully independent measure of performance.

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You already know how to measure how well these algorithms work (way back in our discussion of gene finding!)…

True answer:

		Positive	Negative
Algorithm predicts:	Positive	True positive	False positive
	Negative	False negative	True negative

Specificity = TP / (TP + FP)

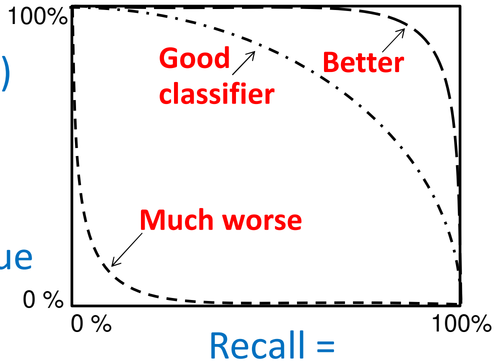
Sensitivity = TP / (TP + FN)

You already know how to measure how well these algorithms work (way back in our discussion of gene finding!)…

Sort the data by their classifier score, then step from best to worst and plot the performance:

Precision = TP / (TP + FP)

also called positive predictive value (PPV)



Recall = TP / (TP + FN) (= sensitivity)

Precision-recall curve

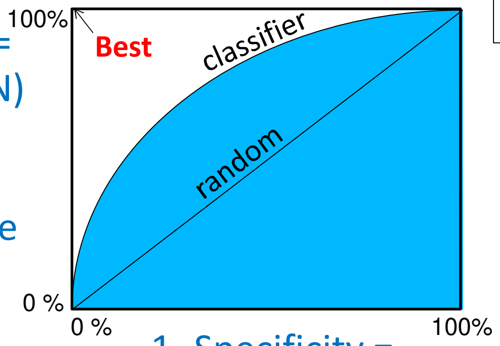
Another good option:

Sort the data by their classifier score, then step from best to worst and plot the performance:

First used in WWII to analyze radar signals (e.g., after attack on Pearl Harbor)

Sensitivity = TP / (TP + FN)

also called True Positive Rate (TPR)



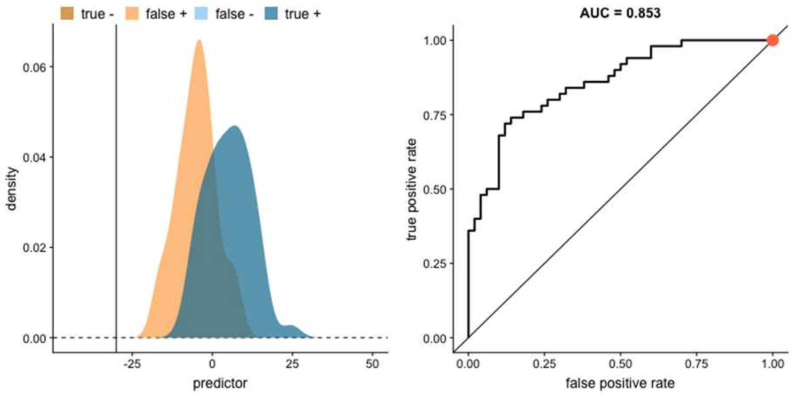
1- Specificity = FP / (FP + TN)

also called False Positive Rate (FPR)

ROC curve

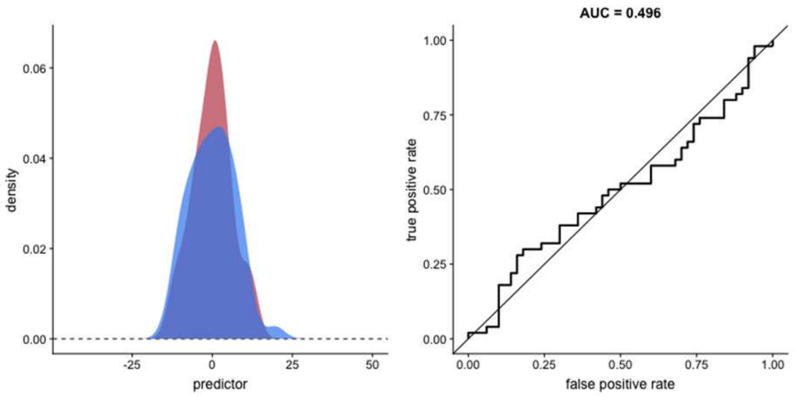
(receiver operator characteristic)

ROC curve, as you go from stronger to weaker predictions



Thanks to Dariya Sydykova (UT Austin), for her excellent visualizations, available here: https://github.com/dariyasydykova/open_projects/tree/master/ROC_animation

ROC curve, as you go from stronger to weaker classifiers



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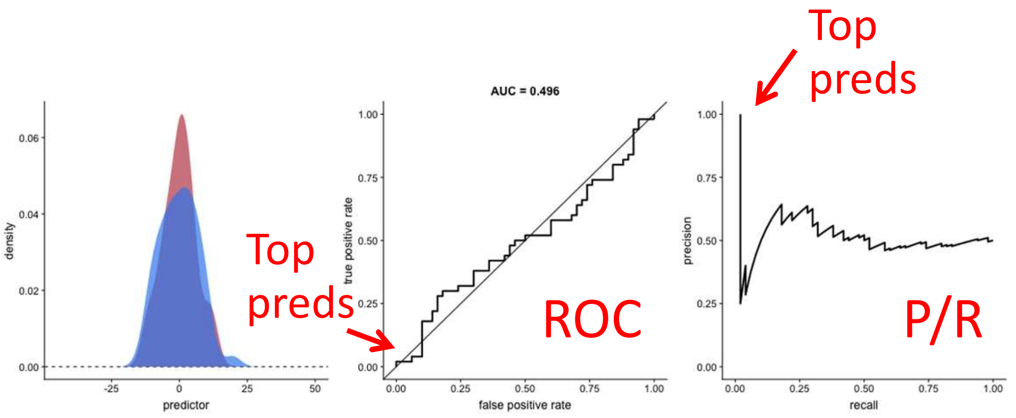
ROC versus Recall/Precision

The 2 measures are related and both useful. They differ strongly in performance as proportions of positive and negative classes change.



Top preds

ROC

Top preds

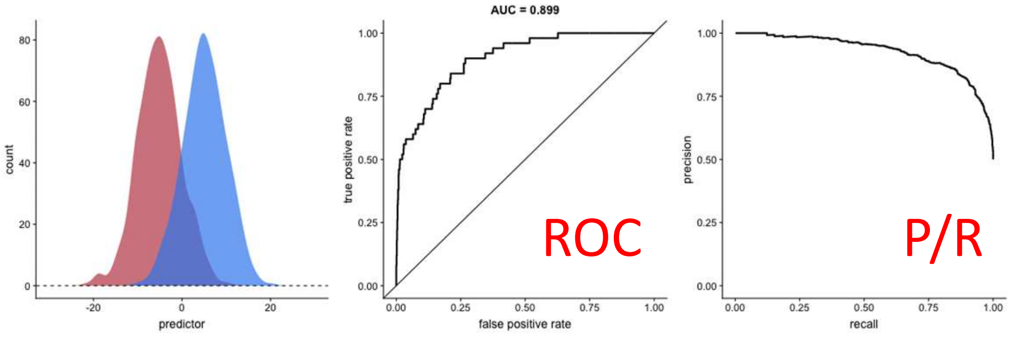
P/R

Thanks to Dariya Sydykova (UT Austin), for her excellent visualizations, available here: https://github.com/dariyasydykova/open_projects/tree/master/ROC_animation

ROC versus Recall/Precision

- R/P depends <u>strongly</u> on relative rates of the 2 classes
- ROC performance is <u>independent</u> of their relative rates

(It may be important or not for your particular problem…)



ROC

P/R

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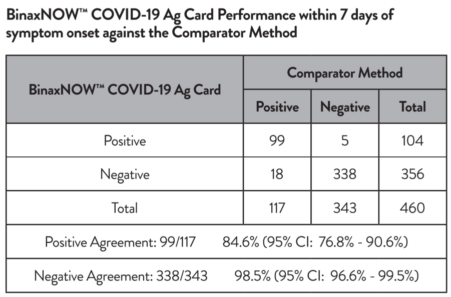
For example, the FDA evaluates diagnostic tests (≈classifiers) using ROC-like measures



BinaxNOW™ COVID-19 Ag Card Performance within 7 days of symptom onset against the Comparator Method

BinaxNOW™ COVID-19 Ag Card	Comparator Method		
	Positive	Negative	Total
Positive	99	5	104
Negative	18	338	356
Total	117	343	460
Positive Agreement: 99/117	84.6% (95% CI: 76.8% - 90.6%)		
Negative Agreement: 338/343	98.5% (95% CI: 96.6% - 99.5%)		

Sensitivity (TPR)

1- Specificity (FPR)

Abbott BinaxNOW™ COVID-19 Ag CARD

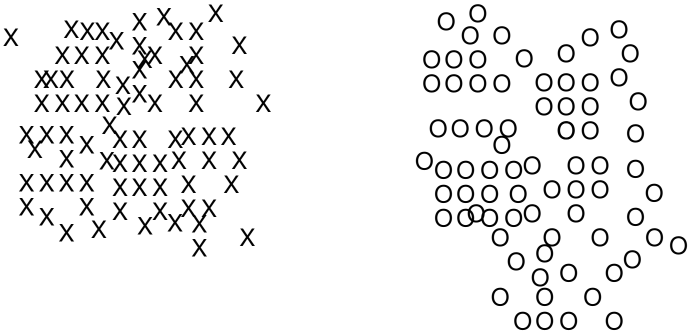
Any guesses why?

Hint: How would COVID test performance change for ROC vs Precision/Recall as the infection rate in the population changes?

https://www.fda.gov/media/141570/download

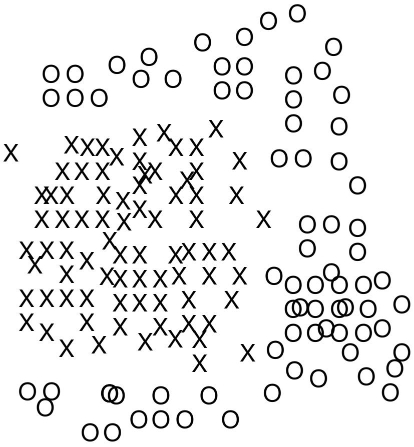
Back to our minimum distance classifier…

Would it work well for this data?



Back to our minimum distance classifier…

How about this data? What might?



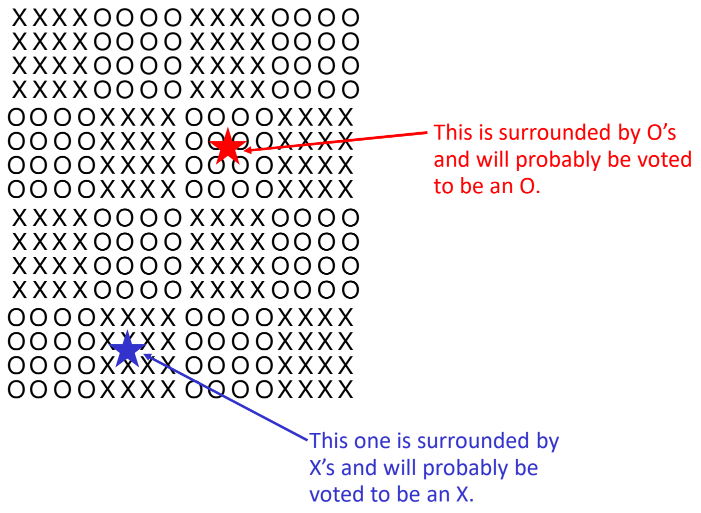
Back to our minimum distance classifier…

How about this data? What might?

```
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
```

This is a great case for something called
a ***k-nearest neighbors classifier***:

For each new object, calculate the *k* closest data points.
Let them vote on the label of the new object.



```
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
XXXXOOOOXXXXOOOO
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
OOOOXXXXOOOOXXXX
```

This is surrounded by O's and will probably be voted to be an O.

This one is surrounded by X's and will probably be voted to be an X.

This is a great case for something called
a ***k-nearest neighbors classifier***:

For each new object, calculate the *k* closest data points.
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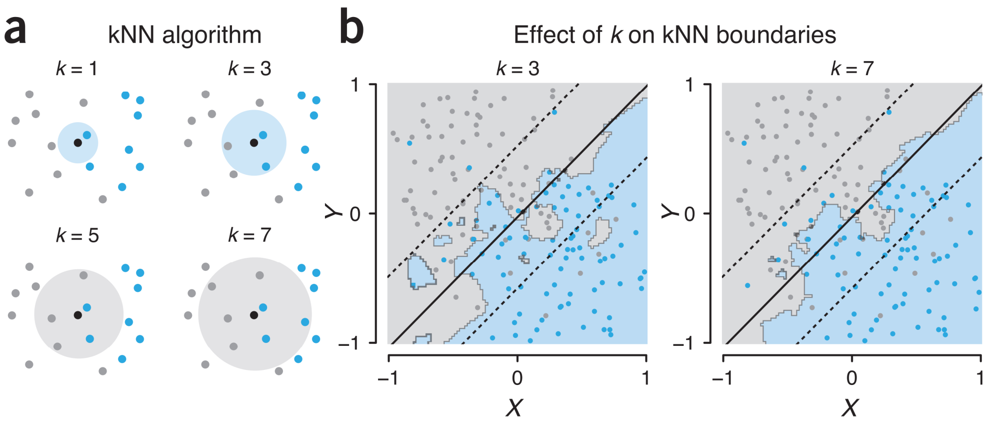

kNN can (and often will) have complex, non-linear decision boundaries

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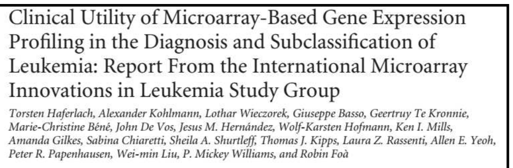
Back to leukemias. There was a follow-up study in 2010:



Clinical Utility of Microarray-Based Gene Expression Profiling in the Diagnosis and Subclassification of Leukemia: Report From the International Microarray Innovations in Leukemia Study Group

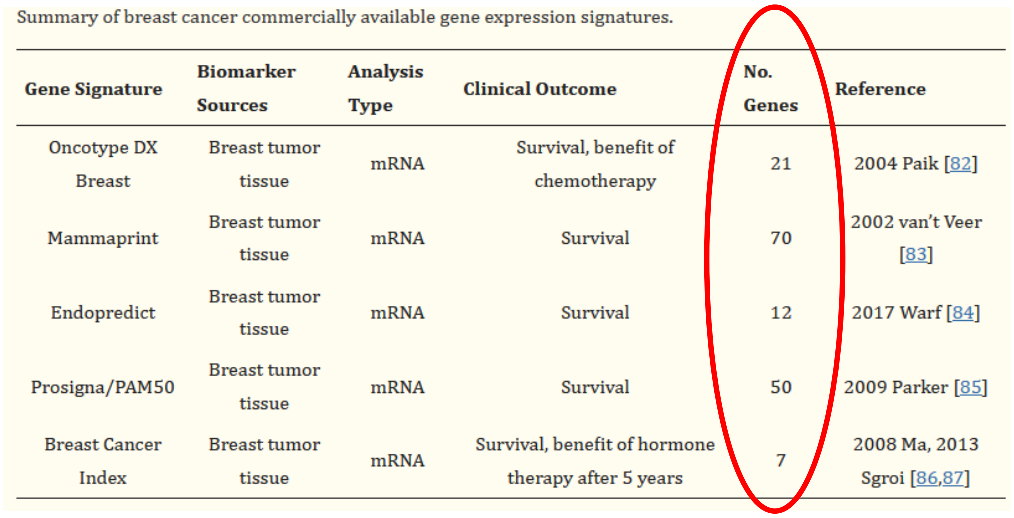
Torsten Haferlach, Alexander Kohlmann, Lothar Wieczorek, Giuseppe Basso, Geertruy Te Kronnie, Marie-Christine Béné, John De Vos, Jesus M. Hernández, Wolf-Karsten Hofmann, Ken I. Mills, Amanda Gilkes, Sabina Chiaretti, Sheila A. Shurtleff, Thomas J. Kipps, Laura Z. Rassenti, Allen E. Yeoh, Peter R. Papenhausen, Wei-min Liu, P. Mickey Williams, and Robin Foà

- **Tested clinical use of expression profiling to subtype leukemias**
- **Meta-analysis of 11 labs, 3 continents, 3,334 patients**
- **Stage 1 (2,096 patients):**
  92.2% classification accuracy for 18 leukemia classes (99.7% median specificity)
- **Stage 2 (1,152 patients):**
  95.6% median sensitivity and 99.8% median specificity for 14 subtypes of acute leukemia
- **Microarrays outperformed routine diagnostics in 29 (57%) of 51 discrepant cases**

Conclusion: "Gene expression profiling is a robust technology for the diagnosis of hematologic malignancies with high accuracy"

J Clin Oncol 28:2529-2537. © 2010

Current commercial breast cancer gene expression panels use this same strategy



Summary of breast cancer commercially available gene expression signatures.

Gene Signature	Biomarker Sources	Analysis Type	Clinical Outcome	No. Genes	Reference
Oncotype DX Breast	Breast tumor tissue	mRNA	Survival, benefit of chemotherapy	21	2004 Paik [82]
Mammaprint	Breast tumor tissue	mRNA	Survival	70	2002 van't Veer [83]
Endopredict	Breast tumor tissue	mRNA	Survival	12	2017 Warf [84]
Prosigna/PAM50	Breast tumor tissue	mRNA	Survival	50	2009 Parker [85]
Breast Cancer Index	Breast tumor tissue	mRNA	Survival, benefit of hormone therapy after 5 years	7	2008 Ma, 2013 Sgroi [86,87]

Prognostic Cancer Gene Expression Signatures: Current Status and Challenges (2021) *Cells* 10(3): 648

In practice, if you want to explore classifiers, I also strongly recommend always testing these classifiers:

Random forests
Support vector machines (SVM)

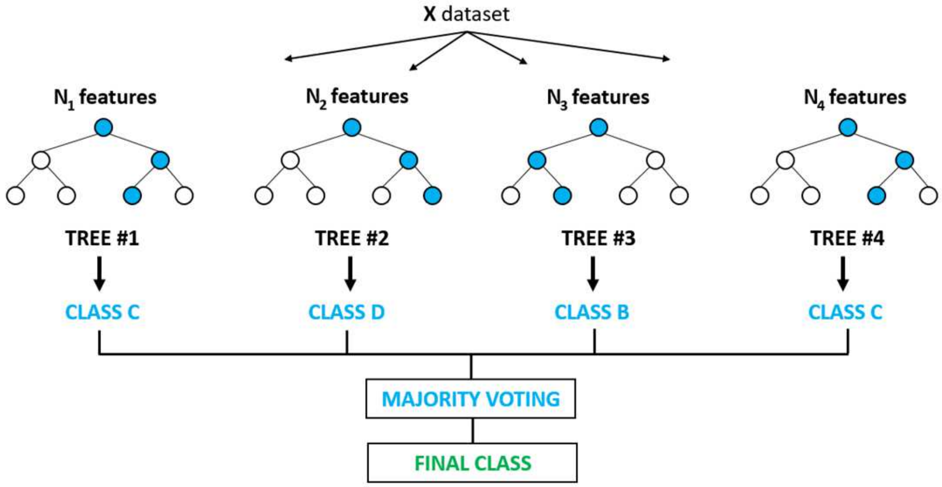
These two are surprisingly often the best for many biological classification problems. Weka can do both of them.

→ Note that I didn't say neural networks. Deep neural networks can be extremely powerful (e.g. AlphaFold) but usually require extensive training examples. In general, you'll often be better off starting off with the above classifiers for many problems, only moving to deep neural networks if you really need to and when you have data to support it.
→ We'll talk about NNs in the next 2 lectures, including large language models.

The two-slide overview of **Random forest classifiers:**

(1) Construct many decision trees from random subsets of your features. Because the features vary across trees, trees tend to be weak but uncorrelated

(2) All the trees "vote" on the answer, majority wins.



https://www.globalsoftwaresupport.com/random-forest-classifier-bagging-machine-learning/

The two-slide overview of **Random forest classifiers:**

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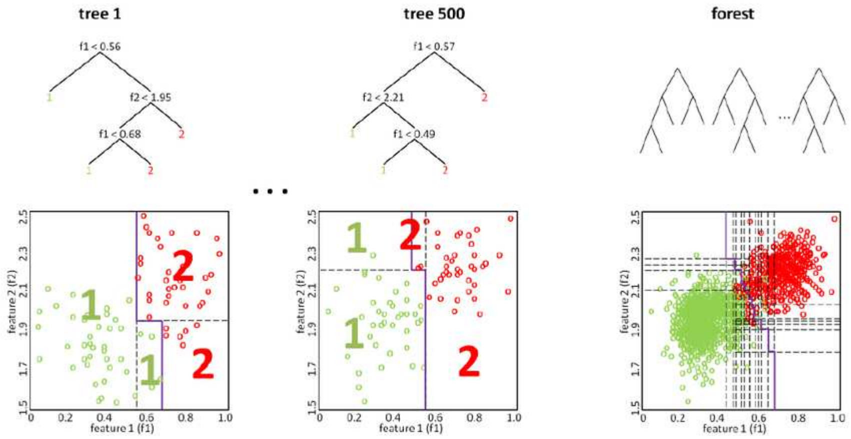
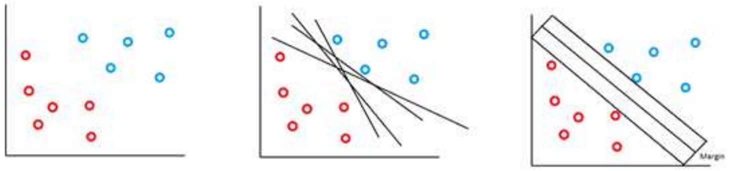


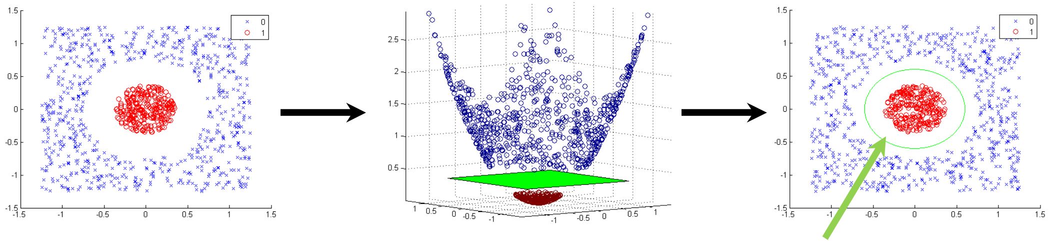
https://www.researchgate.net/figure/The-Random-Forest-classifier-is-an-ensemble-of-decision-trees-where-the-single-trees-are_fig1_228540194

The one-slide overview of **Support vector machines:**

(1) Goal: make a linear classifier, choosing a decision boundary that *maximizes* the *distance margin* between classes



(2) But what if the boundary is non-linear? Use ***kernels*** to implicitly map the data to higher dimension where a linear decision can be made



https://quantdare.com/svm-versus-a-monkey/

Maximum margin hyperplane

In practice, if you want to explore classifiers, I strongly recommend the Weka package:

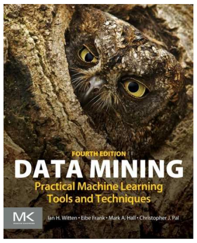
http://www.cs.waikato.ac.nz/ml/weka/

WEKA
The University of Waikato

It’s free, and easy to install, use, & troubleshoot. It lets you quickly test many alternative (well-vetted) classifiers,
all in a proper cross-validated/precision-recall framework.

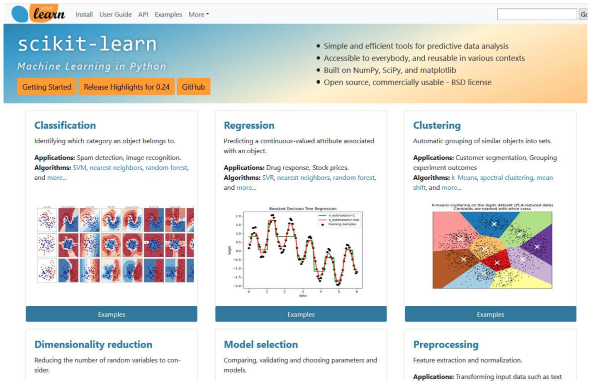
Here’s a nice step-by-step intro for biologists :
Introducing Machine Learning Concepts with WEKA, in *Statistical Genomics, Methods in Molecular Biology,* v. 1418, p. 353-378, 24 March 2016

http://link.springer.com/content/pdf/10.1007%2F978-1-4939-3578-9_17.pdf


FOURTH EDITION
DATA MINING
Practical Machine Learning Tools and Techniques
MK
Ian H. Witten • Eibe Frank • Mark A. Hall • Christopher J. Pal

There’s also a great book to walk you through the entire process.
Highly recommended!!!

In Python, you can also use the scikit-learn library:
https://scikit-learn.org/stable/
Like Weka, it’s free, easy to install & use, and very powerful


scikit learn
Install
User Guide
API
Examples
More
Go
Getting Started
Release Highlights for 0.24
GitHub
Boosted Decision Tree Regression
n_estimators=1
n_estimators=300
training samples
target
data
K-means clustering on the digits dataset (PCA-reduced data)
Centroids are marked with white cross
Examples
Examples
Examples

scikit-learn

Machine Learning in Python

- Simple and efficient tools for predictive data analysis
- Accessible to everybody, and reusable in various contexts
- Built on NumPy, SciPy, and matplotlib
- Open source, commercially usable - BSD license

Classification

Identifying which category an object belongs to.

Applications: Spam detection, image recognition.
Algorithms: SVM, nearest neighbors, random forest, and more...

Regression

Predicting a continuous-valued attribute associated with an object.

Applications: Drug response, Stock prices.
Algorithms: SVR, nearest neighbors, random forest, and more...

Clustering

Automatic grouping of similar objects into sets.

Applications: Customer segmentation, Grouping experiment outcomes
Algorithms: k-Means, spectral clustering, mean-shift, and more...

Dimensionality reduction

Reducing the number of random variables to consider.

Model selection

Comparing, validating and choosing parameters and models.

Preprocessing

Feature extraction and normalization.

Applications: Transforming input data such as text

I recommend combining it with the Pandas library for data analysis to make it easy to work with big, tabular datasets:
https://pandas.pydata.org/

Coming up:

The next two lectures will be guest lectures covering the basics of deep neural networks, with talks on

Protein 3D structural modeling and prediction w/ AlphaFold/ChimeraX

DeepNNs, Large Language Models, & ESM