High-Throughput Sequencing Course

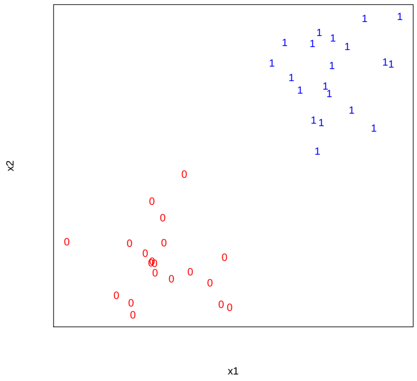
Supervised Learning

Biostatistics and Bioinformatics

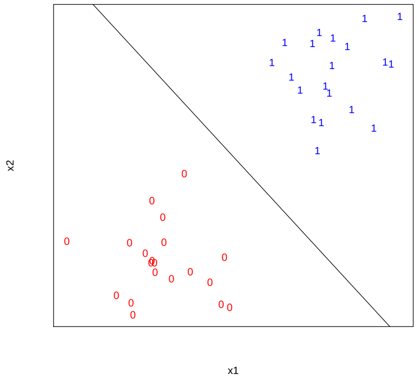
Summer 2017

Duke University School of Medicine

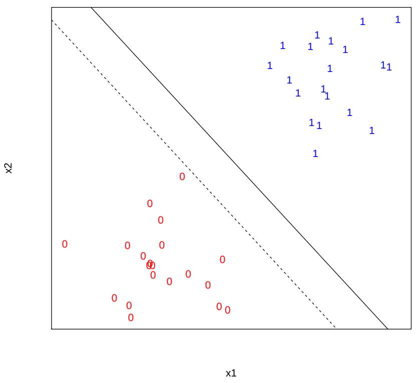
Classification Problem



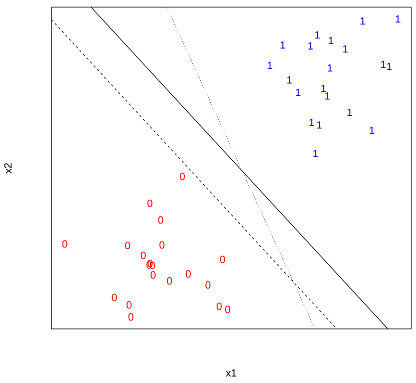
Clear-cut case



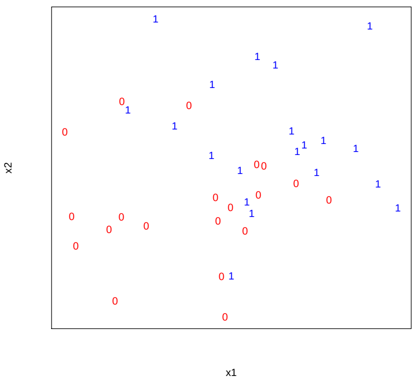
Clear-cut case?



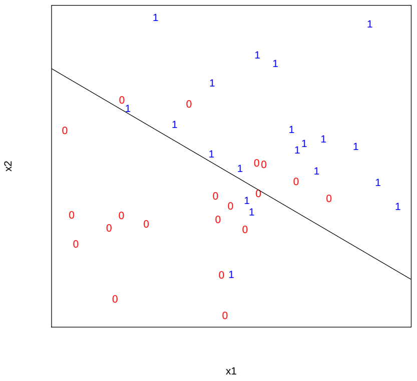
Clear-cut case??



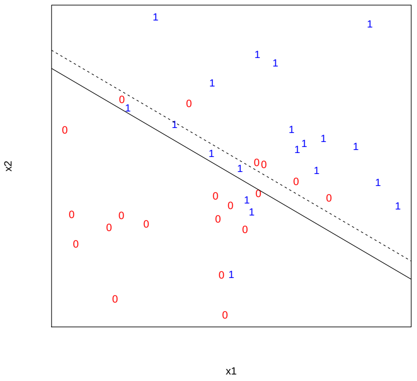
Less Clear-cut case



Less Clear-cut case



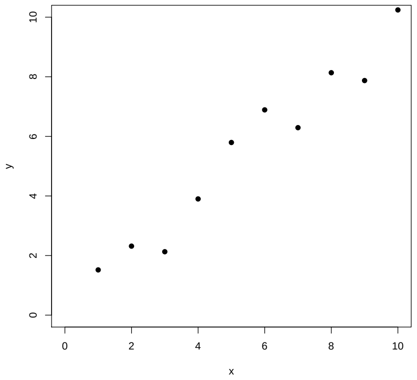
Less Clear-cut case



Regression Problem

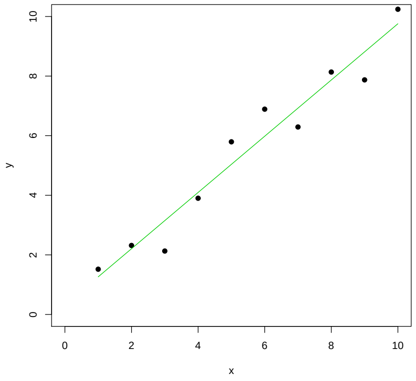
```
set.seed(10) x <- 1:10 y = x + rnorm(10, 0.5)

par(mfrow = c(1, 1), bg = "white") plot(x, y, xlim = c(0, 10), ylim = c(0, 10), pch = 19)
```

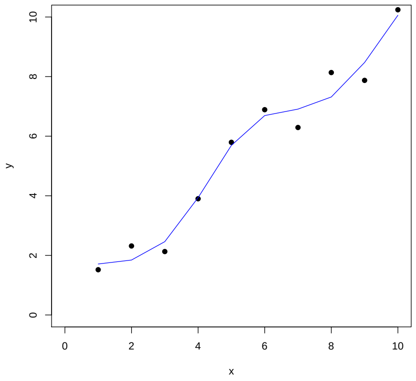


Linear Regression (lin)

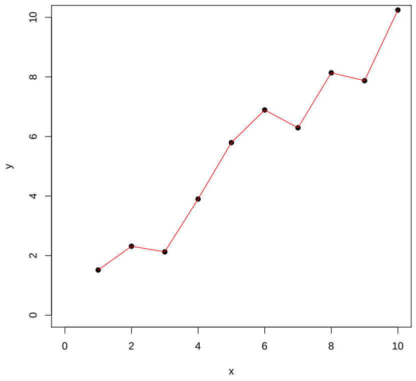
```
par(mfrow = c(1, 1), bg = "white") plot(x, y, xlim = c(0, 10), ylim = c(0, 10), pch = 19) modlm =
lm(y   x) lines(x, predict(modlm), col = 3)
```



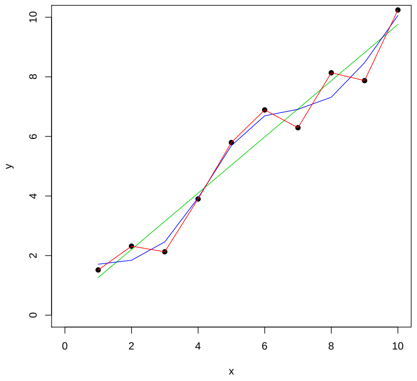
Spline Regression (spl)



Connect the dots (ctd)



Which Approach?



Supervised Learning (Classification)

- Goal: Predict a binary outcome (Y) on the basis of baseline information (X)
- Y assumes the value 0 or 1 (e.g., control vs case, or AML vs ALL)
- X could be single variable or be a vector of multiple variables
- Example: Can you predict Y on the basis of two genes say X_1 and X_2
- Note that a goal is to build a machine that will take on two values X_1 and X_2 and return a 0 or a 1
- You can denote this machine as a function $g(x_1, x_2)$

Classifier

- We will denote the predictor or classifier by $g(x)$
- $x = (x_1, x_2)$ is the vector of gene expressions for genes 1 and 2
- Based on x, the classifier g makes a prediction for the outcome
- Note that $g(x) = 0$ or $g(x) = 1$
- The prediction is *correct* if $Y = 1$ and $g(x_1, x_2) = 1$, or $Y = 0$ and $g(x_1, x_2) = 0$
- The prediction is *wrong* if $Y = 0$ and $g(x_1, x_2) = 1$, or $Y = 1$ and $g(x_1, x_2) = 0$

Prediction Assessment

	$g(x_1, x_2) = 0$	$g(x_1, x_2) = 1$
$Y = 0$	True-Negative	False-Negative
$Y = 1$	False-Negative	True-Positive

Steps to Construct a Classifier

- Collect a random data set of size n to build (train) a classifer
- This is called the training data
- On the basis of these data, construct the classifier g_n
- It is subscripted by n to emphasize that it is trained on the basis of the training data
- Note that the final performance of g_n is *not* be judged on the basis of the training data
- It is to be judged on the basis of its performance on *future* data
- Called testing data

Steps in Notation

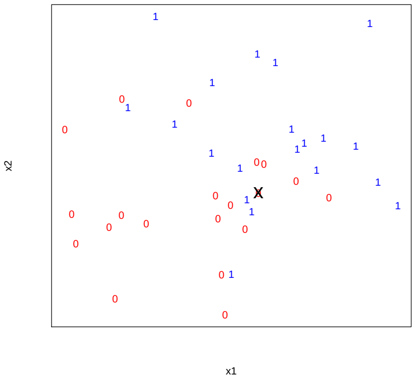
- Collect the training data $(X_1, Y_1), \ldots, (X_n, Y_n)$
- Construct a classifier g_n on the basis of the training data
- Apply g_n to a new data set $X_1^*, \ldots, X_k^*$ to get
- k predictions: $\hat{Y}_1^*, \ldots, \hat{Y}_k^*$
- Compare the predictions to the observed outcomes $Y_1^*, \ldots, Y_k^*$
- Note that at the testing stage, you are blinded to the Y_k^*

k-Nearest Neighborhood (Non-parametric)

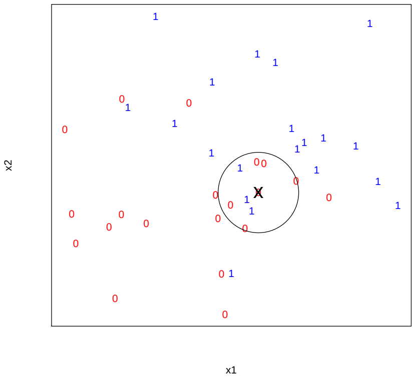
- Generally, non-parametric methods (e.g., k-NN) are preferred
- These do not make strong assumptions on the specific shape of the underlying relationship, if there is one, between X and Y
- For each x (point on the scatter plot), identify the k nearest neighbors
- Among the k neighbors, count the number of responders (say r_x)
- Set

$$\hat{\eta}(x) = \frac{r_x}{k}$$

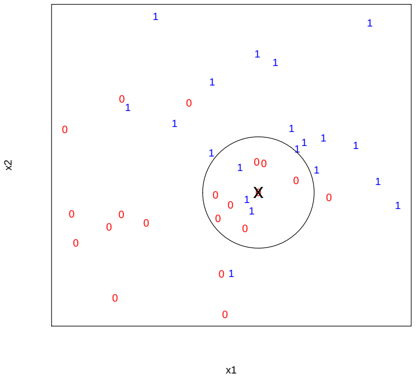
k-Nearest Neighborhood



3-Nearest Neighborhood



5-Nearest Neighborhood



Mean Regression Model

- $E(Y)$ is the *unconditional* (on X) mean of Y.
- Model the mean relationship between Y and X

$$\eta(x) = E(Y|X = x)$$

- $\eta(x)$ is the *conditional* (on X) mean of Y given that X has realized the value x.

Bayes Classifier

$$g(X) = \begin{cases} 1 & \text{if } \eta(X) \geq \frac{1}{2}, \\ 0 & \text{if } \eta(X) < \frac{1}{2} \end{cases}$$

- This classifier is ”optimal” in the sense that there is no better classifier with respect to minimizing the error ($P(g(X) \neq Y)$).
- Suppose that g^* is another classifier. Then

$$P(g(X) \neq Y) \leq P(g^*(X) \neq Y)$$

- Note that the optimality concerns $\eta(x)$ and not $\hat{\eta}(x)$.

Logistic Regression (Parametric)

- Most commonly used method for modeling the relationship between a binary response and a set of co-variables

$$\text{logit}(\eta(x)) = \beta_0 + \beta_1 x_1 + \beta_2 x_2,$$

where

$$\text{logit}(\eta(x)) = \log\left(\frac{p}{1-p}\right),$$

for $p \in (0, 1)$ is called the ”logit” function.

Estimating $\eta(x)$

- Estimate the model parameters (β_0, β_1 and β_2) using maximum-likelihood estimation to get $\hat{\beta}_0, \hat{\beta}_1$ and $\hat{\beta}_2$
- For the logistic model

$$\hat{\eta}(x) = \frac{\exp(\hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_2)}{1 + \exp(\hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_2)}$$

Other Classification Methods

- Fisher’s Linear Discriminant
- Support Vector Machines (SVM)
- Classification and Regression Trees (CART)
- Random Forests (aggregated trees)
- Methods for ”Deep” learning

Bias versus Variance

- A very important principle in statistical modeling is the so called *bias-variance tradeoff*
- The bias of $\hat{\eta}(x)$ is

$$b(x) = \hat{\eta}(x) - \eta(x)$$

- The variance of $\hat{\eta}(x)$ is

$$v(x) = E(\hat{\eta}(x) - \eta(x))^2)$$

- The bias-variance tradeoff implies that both cannot be minimized simultaneously
- For example for the k-NN method increasing k increases bias while decreasing variance

Training and Testing

- In practice, the model is first estimated (trained) using an initial set of data
- This data set is usually called the ”training” data
- Once the model is trained, then it is applied to an ”independent” set of data
- This data set is usually called the ”testing” (or validation) data set

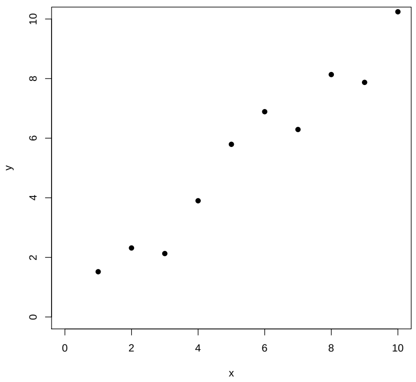
Parsimony

- The model should be parsimonious (less is more)
- Including too many noisy/unimportant features often degrades the performance of the classifier.
- Including highly dependent induces problems (e.g., multi-collinearity from simple linear regression).
- Additional complication: It is not practically/computationally feasible to include tens of thousands of features in the model.

Overfitting

- Two many parameters compared to the number of data points in the training set
- A complicated model will fit the training set well
- It will however perform poorly for an independent set.

Overfitting



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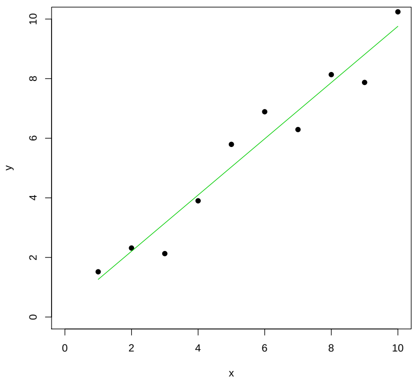
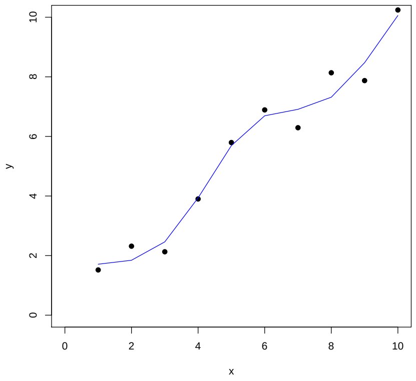
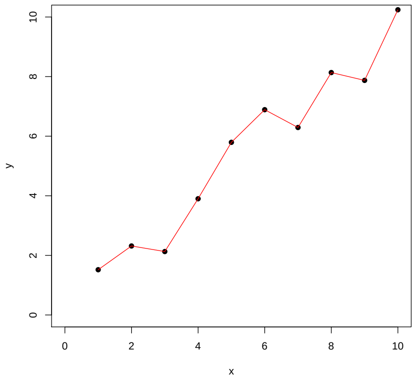


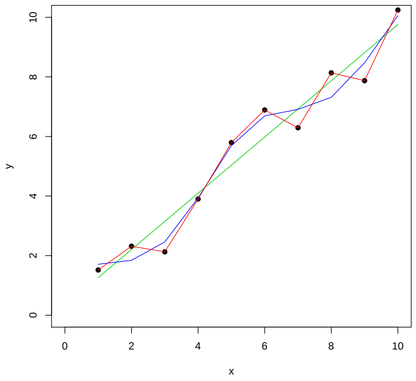
Spline Regression (spl)



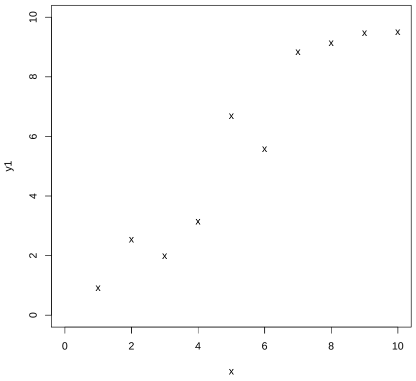
Connect the dots (ctd)



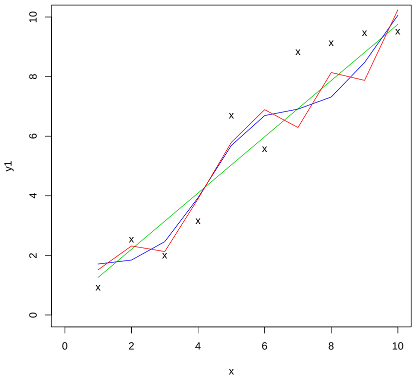
RSS: 4.1 (lin) vs 1.9 (spl) vs 0 (ctd)



New Data Set



RSS: 11 (lin) vs 12.4 (spl) vs 14 (ctd)



Two Challenges in Building a Classifier

1. Feature Selection:
   - It is neither feasible nor provident to build a classifier based on all available variables
   - A subset of the variables has to be selected to build the model
   - This is also called feature extraction
2. Tuning Parameter Selection:
   - Statistical methods may have one or more parameters that have to be set
   - For example when using k-NN, one has to decide what k should be (e.g., 1, 3 or 5 or how about 8)?
   - Choosing the defaults set by the software is inappropriate
   - The feature selection method could also have tuning parameters that have to be set (e.g., the number of features to be selected)
   - The performance of the method could be highly sensitive to the choice of these parameters

Feature Selection

- Reasonable Feature Selection is *critical* if not the most important component of model building.
- You cannot expect to build a good model if you select poor features.
- This is also called Feature Extraction
- We will talk about a few approaches that have been used in the literature.

Feature Selection (ranked based on test-statistic)

- Compute the two-sample t-test for all m features (based on the training set)
- Identify the top say 10 or 15 features (e.g, ranked based on the absolute value of the test statistic).
- Build a model on these ”top” features (based on the training set)
- Alternatively, you could select all features for which the P-value is less than a certain threshold (say 0.001).
- You can also use the Wilcoxon rank sum statistic to protect against choosing features with outliers.

Feature Selection (Ordination Methods)

- A standard approach for reducing the dimension in the microarray setting is the method of Principal Components (PCs)
- The PCs are combinations of the original variables (gene expressions) that have maximum variability
- The are also constructed as to be uncorrelated with another
- This attempts to address the issue of high dimension and multi-collinearity simultaneously.
- One can use the principal components (say the first two or three) as the features
- Alternatively, one can first reduce the dimension by using the two-sample test-statistic approach and then get the PCs

Tuning

- You cannot expect to be able to build a model using default values provided by the software package.
- If you use k-NN you need to decide which k (e.g., 3 or 5 or 7) you want to use
- If you use the simple feature selection method you need to determine how many ”top” features you want to use
- If you are doing PC dimension reduction, you need to determine how many PCs you want to use.
- In some books and articles, ”tuning” only refers to the choice of the model parameter (e.g., k in k-NN)
- Must take a broader perspective as the choices in the FS part also affect the results.

Validation

- Split the data into a training and a mutually exclusive testing set
- Build the model (including feature selection, tuning) on the *training* set
- Evaluate the performance of the model on the *testing set*
- IMPORTANT: The model is built based on the *training* set. The *testing* set should not contribute *any* information.
- Violating this principle will invariably result in bias

Error Substitution Validation

- Error Substitution Validation: The testing set is empty.
- Test the model you just built on the *training* set
- This approach cannot be recommended under any circumstance.
- Analogy: Assess the fit of the linear model by plotting the fitted (from the data) to the observed data.
- A bona-fide testing set is required.
- Will demonstrate how this can lead to noise discovery

Hold-out Method

- Split the data into two parts
- Keep the testing set locked up
- Better yet, ask an ”honest” broker to keep it from you until you are ready to test the model
- This approach is reasonable if you have a large number of cases
- It may be problematic if the outcomes are sparse

k-fold Cross-Validation

- Many microarray experiments are from smaller (e.g., pilot) studies
- It is not impossible to get reasonably size training and testing sets this cases
- A reasonable approach to get around this is k-fold cross-validation (CV)
- Randomly split cases into k (nearly) equally sized subsets (folds).
- At each step take of these k portions as the *testing* set and construct the *training* set based on the other $k-1$ portions
- Special case is Leave-One-Out CV (LOOCV) where $k = n$
- For really small data sets, LOOCV is often the best (most practical) choice.

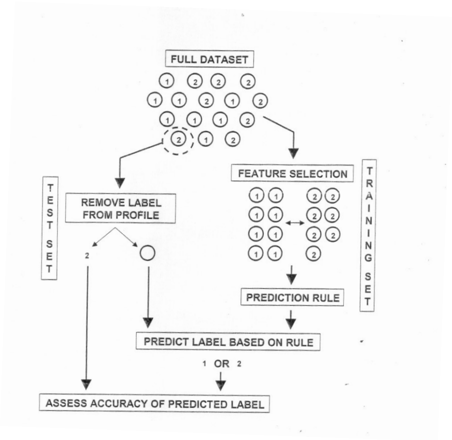
Naive Cross-Validation

- Naive Validation: Do the feature selection once based on all n cases
- In each CV step use the same set of features.
- This will invariably make the results look better than they really are
- It should be avoided unless one feels *very* certain about the features (say biologically relevant gathered *a priori*

Proper Cross-Validation

- Choose the first fold and set it aside the other $k-1$ folds
- Carry out Feature Selection on the other $k-1$ folds
- Train the model based the top features on the $k-1$ folds
- Test the model on the first fold left out
- Repeat the above for the second fold (set aside the second fold, leave in the first and the next $k-2$ folds).

Important Illustration (Fig 8.5) from Simon et al.


FULL DATASET
TEST SET
REMOVE LABEL FROM PROFILE
FEATURE SELECTION
TRAINING SET
PREDICTION RULE
PREDICT LABEL BASED ON RULE
1 OR 2
ASSESS ACCURACY OF PREDICTED LABEL

Simulate Data for k-NN Prediction

- Simulate expression from 1000 genes for 40 patients. Let the first 20 be responders and the remaining 20 be non-responders

```
set.seed(123)
n = 20
m = 1000
EXPRS = matrix(rnorm(2 * n * m), 2 * n, m)
rownames(EXPRS) = paste("pt", 1:(2 * n), sep = "")
colnames(EXPRS) = paste("g", 1:m, sep = "")
grp = rep(0:1, c(n, n))
```

- Pick the top 10 features based on the two-sample t-test

```
stats = abs(rowttests(t(EXPRS), factor(grp))$statistic)
ii = order(-stats)
```

- Filter out all genes except the top 10

```
TOPEXPRS = EXPRS[, ii[1:10]]
```

Error Resubstitution and Naive CV

- Error resubstitution (Training and Testing set are the same)

```
mod0 = knn(train = TOPEXPRS, test = TOPEXPRS, cl = grp, k = 3)
table(mod0, grp)

##     grp
## mod0  0  1
##    0 17  0
##    1  3 20
```

- Cross-validated predictions (the features selection is not part of the CV process)

```
mod1 = knn.cv(TOPEXPRS, grp, k = 3)
table(mod1, grp)

##     grp
## mod1  0  1
##    0 16  0
##    1  4 20
```

- Note that in both examples, `TOPEXPR` not `EXPR` is used.

R Function to Implement Proper CV based on k-NN

```
top.features <- function(EXP, resp, test, fsnum) {
    top.features.i <- function(i, EXP, resp, test, fsnum) {
        stats <- abs(mt.teststat(EXP[, -i], resp[-i], test = test))
        ii <- order(-stats)[1:fsnum]
        rownames(EXP)[ii]
    }
    sapply(1:ncol(EXP), top.features.i, EXP = EXP, resp = resp, test = test,
        fsnum = fsnum)
}


# This function evaluates the knn

knn.loocv <- function(EXP, resp, test, k, fsnum, tabulate = FALSE, permute = FALSE) {
    if (permute)
        resp = sample(resp)
    topfeat = top.features(EXP, resp, test, fsnum)
    pids = rownames(EXP)
    EXP = t(EXP)
    colnames(EXP) = as.character(pids)
    knn.loocv.i = function(i, EXP, resp, k, topfeat) {
        ii = topfeat[, i]
        mod = knn(train = EXP[-i, ii], test = EXP[i, ii], cl = resp[-i], k = k)[1]
    }
    out = sapply(1:nrow(EXP), knn.loocv.i, EXP = EXP, resp = resp, k = k, topfeat = topfeat)
    if (tabulate)
        out = ftable(pred = out, obs = resp)
    return(out)
}
```

Proper Cross-Validation

- Finally, we conduct proper cross-validation using the previous `R` function
- At each iteration, the top 10 features are selected based on the data from the $n - 1$ samples in the training set

```
knn.loocv(t(EXPRS), as.integer(grp), "t.equalvar", 3, 10, TRUE)

##      obs  0  1
## pred
## 0         7  7
## 1        13 13
```

- Note that `EXPRS` not `TOPEXPR` is used.
- The classification rate is 50% (as expected)

Naive LOOCV: Quantitative trait

- Repeat the last experiment with a noisy quantitative outcome
- First simulate a data matrix of dimension $n = 50$ (patients) and m (genes)
- Next draw the outcome for $n = 50$ patients from a standard normal distribution independent of the data matrix
- There is no relationship between the expressions and the outcome (by design)
- We consider $m = 45$ and $m = 50000$
- We conduct Naive LOOCV using the top 10 features

Naive LOOCV: Quantitative trait

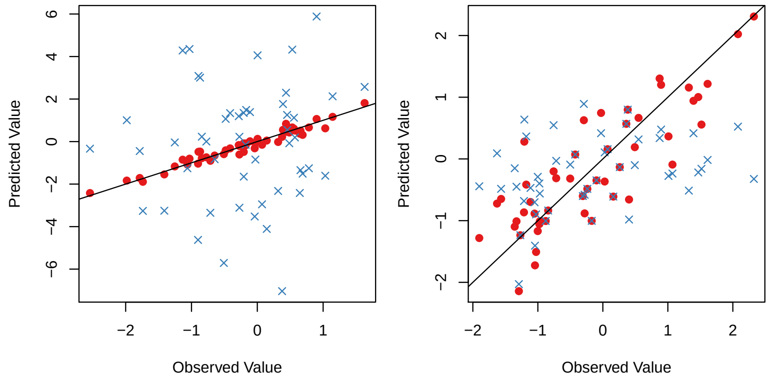


Figure taken from Owzar *et al*; *Clin Transl Sci* 2011.

Training, Validation and Testing Approach

- Before you test the model, you must freeze it
- You may want to split the Training set further into a Training and Validation set
- Use the Validation set to ”tune” the model.

Final Remarks

- It is OK to try different methods (other classifiers, feature selection or tuning methods)
- Keep track of what you have done and report it (brief description in the paper and details in supplementary material)
- Be careful if you have too few responders
- You could have a model that will classify most patients as a non-responder.
- In this case a 00 ($Y = 0$ and $g(X) = 0$) may not be bona-fide true-negative
- The gold-standard for model validation, is to follow up the cross-validatiion by permutation resampling
- The `R` function provided can be used for this purpose

Pre-processing Challenge

- The X profiles from the testing set need to be ”compatible” to those used to train the model
- In classical experiments with a few biomarkers, the labs had internal controls to ensure that the measurements were properly normalized
- For RNA-Seq data, you observe counts (not expressions)
- Number of reads mapped to genes are *not* comparable
- Why?
- The current ”state” of the art is to ”normalize” the counts into expressions
- This is a practical but not rigorous solution
- One has to up the ante if the classifier is to be used for important decision (e.g., treating a patient with a toxic but potentially effective drug)

On Data and Answers

”The data may not contain the answer. The combination of some data and an aching desire for an answer does not ensure that a reasonable answer can be extracted from a given body of data.”

John Wilder Tukey