

## Introduction to Random Forests for Gene Expression Data

Utah State University – Spring 2014  
STAT 5570: Statistical Bioinformatics  
Notes 3.5

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## References

- Breiman, Machine Learning (2001) 45(1): 5-32.
- Diaz-Uriarte and Alvarez de Andres, BMC Bioinformatics (2006) 7:3.
- Cutler, Cutler, and Stevens (2012) Random Forests. In Zhang and Ma, editors, *Ensemble Machine Learning: Methods and Applications*, pp. 157-175.

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## Gene Profiling / Selection

- “Observe” gene expression in different conditions – healthy vs. diseased, e.g.
- Use simultaneous expression “profiles” of thousands of genes (what are the genes doing across arrays)
- Look at which genes are “important” in “separating” the two conditions; i.e., what determines the conditions’ “signatures”

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## Machine Learning

- Computational & statistical inference processes:  
observed data → reusable algorithms for prediction
- Why “machine”?  
want minimal: human involvement
- Why “learning”?  
develop ability to predict
- Here, supervised learning:  
use knowledge of condition type

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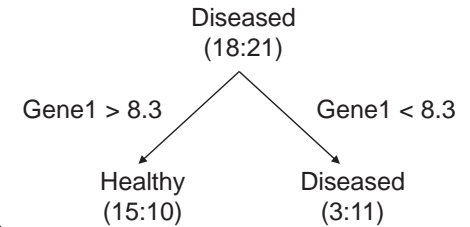
## Machine Learning Methods

- Neural Network
- SVM (Support Vector Machine)
- RPART (Recursive PARTitioning and Regression Trees)
- CART (Classification and Regression Trees)
- Ensembling Learning (average of many trees)
  - Boosting (Shapire et al., 1998)
  - Bagging (Breiman, 1996)
  - RandomForests (Breiman, 2001; Cutler & Stevens 2006; Cutler, Cutler, & Stevens 2008)

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## CART: Classification and Regression Trees

- Each individual (array) has data on many predictors (genes) and one response (disease state)
- Think of a tree, with splits based on levels of specific predictors
- Choose predictors and split levels to maximize “purity” in new groups; the best split at each node
- Prediction made by: passing test cases down tree



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## CART generalized: Random Forests

- Rather than using all predictors and all individuals to make a single tree, make a forest of many (**n**tree) trees, each one based on a random selection of predictors and individuals
- Each tree is fit using a bootstrap sample of data (draw with replacement) and ‘grown’ until each node is ‘pure’
- Each node is split using the best among a subset (of size **m**try) of predictors randomly chosen at that node (default is sqrt. of # of predictors) (special case using all predictors: bagging)
- Prediction made by aggregating across the forest (majority vote or average)

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## How to measure “goodness”?

- Each tree fit on a “training” set (bootstrap sample), or the “bag”
- The left-over cases (“out-of-bag”) can be used as a “test” set for that tree (usually 1/3 of original data)
- The “out-of-bag” (OOB) error rate is the: % misclassification

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## What does RF give us?

- Kind of a “black box”
  - but can look at “variable importance”
- For each tree, look at the OOB data:
  - Permute values of predictor  $j$  among all OOB cases
  - Pass OOB data down the tree, save the predictions
  - For case  $i$  of OOB and predictor  $j$ , get:
    - OOB error rate with variable  $j$  permuted –
    - OOB error rate before permutation
- Average across forest to get overall variable importance for each predictor  $j$

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## Why “variable importance”?

- Recall: identifying conditions’ signatures
- Sort genes by some criterion
- Want smallest set of genes to achieve good diagnostic ability

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## Recall – ALL Data

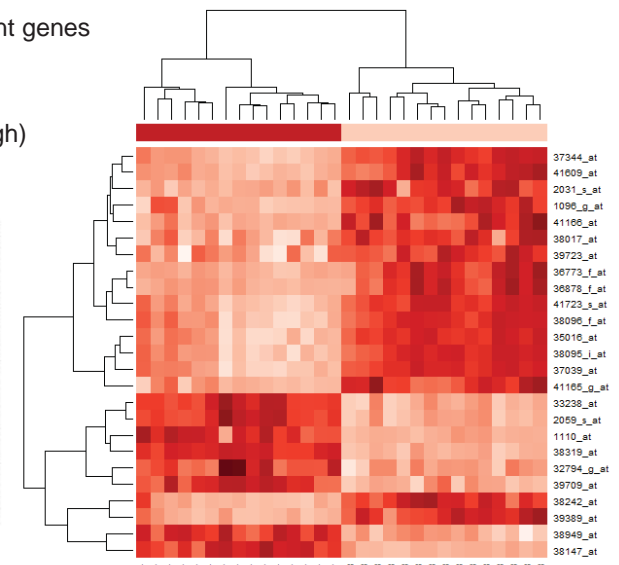
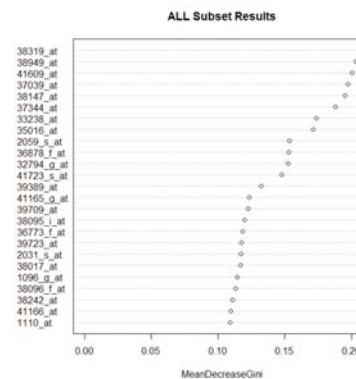
- RMA-preprocessed gene expression data
- Chiaretti et al., Blood (2004) 103(7)
- 12625 genes (hgu95av2 Affymetrix GeneChip)
- 128 samples (arrays)
- phenotypic data on all 128 patients, including:
  - 95 B-cell cancer
  - 33 T-cell cancer

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ALL subset: 4,400 genes, 30 arrays  
(15 B, 15 T)

Look at top 25 most important genes from Random Forest.

Color scale:  
purple (low) to orange (high)



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### First prepare objects for RF
### (similar to slide 13 of Notes 3.3)

# load data
library(affy); library(ALL); data(ALL)

# (here, filter genes on raw scale, then return to log scale)
# also, keep 30 arrays here JUST for computational
# convenience (in-class)
library(genefilter); e.mat <- 2^(exprs(ALL)[,c(81:110)])
ffun <- filterfun(pOverA(0.20,100))
t.fil <- genefilter(e.mat,ffun)
small.eset <- log2(e.mat[t.fil,])
dim(small.eset) # 4400 genes, 30 arrays (15 B and 15 T)

group <- c(rep('B',15),rep('T',15))
# group classification, in order

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# One RF
library(randomForest)
set.seed(1234)
print(date())
rf <- randomForest(x=t(small.eset),y=as.factor(group),
ntree=10000)
print(date()) # about 20 seconds

# Make variable importance plot
varImpPlot(rf, n.var=25, main='ALL Subset Results')

# Get names of most important genes
imp.temp <- importance(rf)
t <- order(imp.temp,decreasing=TRUE)
gn.imp <- rownames(imp.temp)[t]
# these are in order most...least important

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# Get expression values for 25 most important genes
gn.25 <- gn.imp[1:25] # vector of top 25 genes, in order
t <- is.element(rownames(small.eset),gn.25)
sig.eset <- small.eset[t,]
# matrix of expression values,
# not necessarily in order of importance

# Make a heatmap, with group differences obvious on plot
# (similar to Notes 2.3)
library(RColorBrewer)
hmcol <- colorRampPalette(brewer.pal(9,"Reds"))(256)
colnames(sig.eset) <- group
# This will label the heatmap columns
csc <- rep(hmcol[50],30)
csc[group=='T'] <- hmcol[200]
# column side color will be dark for T and light for B
heatmap(sig.eset,scale="row", col=hmcol,ColSideColors=csc)

```

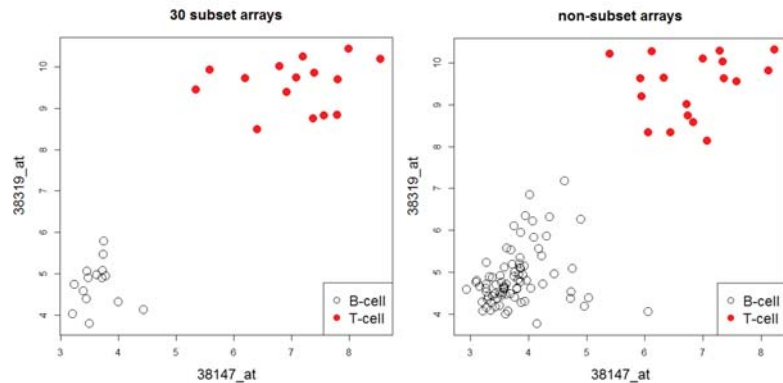
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## Can focus on “variable selection”

- Iteratively fit many forests, each time discarding predictors with low importance from previous iterations
- Use bootstrap to assess standard error of error rates
- Choose the forest with the smallest number of genes whose error rate is within  $u$  standard errors of the minimum error rate of all forests ( $u = 0$  or  $1$ , typically)
- Reference: Diaz-Uriarte and Alvarez de Andres, BMC Bioinformatics (2006) 7:3.
- Online tool: <http://genesrf.bioinfo.cnio.es>

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## RF Variable Selection on ALL subset



Subset: 30 arrays, 4400 genes (non-subset is the remaining 98 arrays)

First forest: 10,000 trees; Subsequent forests: 2,000 trees

At each iteration, drop 20% of variables (genes) until a 2-variable model is built; return the best of the series of models considered.

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# Look at variable selection
library(varSelRF)
set.seed(1234)
print(date())
rf.sel <- varSelRF(t(small.eset), as.factor(group),
  ntree=10000, ntreeIterat=2000, vars.drop.frac=0.2)
print(date()) # 40 seconds
# rf.sel$firstForest is the same as the slide 14 rf object
rf.sig.gn <- rf.sel$selected.vars # "38147_at" "38319_at"
# set.seed(123) above gives genes: "2059_s_at" "38319_at"

# Visualize these two genes
exp.gn.1 <- small.eset[rownames(small.eset)==rf.sig.gn[1],]
exp.gn.2 <- small.eset[rownames(small.eset)==rf.sig.gn[2],]
use.pch <- c(rep(1,15), rep(16,15)) # Define plotting chars.
use.col <- c(rep(1,15), rep(2,15)) # Define plotting colors
plot(exp.gn.1, exp.gn.2, col=use.col, main='30 subset arrays',
  cex.main=1.5, cex.lab=1.5, xlab=rf.sig.gn[1],
  ylab=rf.sig.gn[2], pch=use.pch, cex=2)
legend('bottomright',
  c('B-cell', 'T-cell'), pch=c(1,16), col=c(1,2), cex=1.5)
```

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# Did this overfit these 30 arrays?

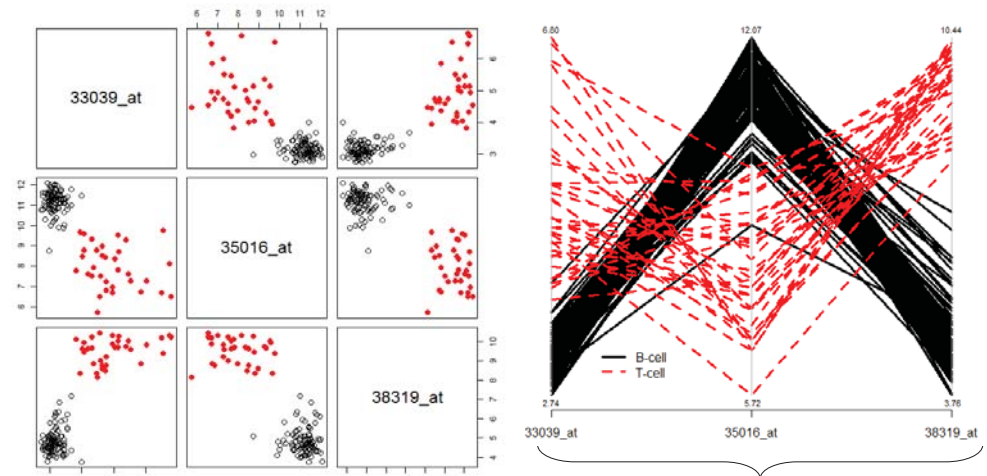
# Look at JUST the other 98

# (first 80 are B-cell, last 18 are T-cell)

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eset.2 <- exprs(ALL)[,c(1:80, 111:128)]
group.2 <- c(rep(0,80), rep(1,18))
exp.gn.1 <- eset.2[rownames(eset.2)==rf.sig.gn[1],]
exp.gn.2 <- eset.2[rownames(eset.2)==rf.sig.gn[2],]
use.pch.2 <- c(rep(1,80), rep(16,18))
use.col.2 <- c(rep(1,80), rep(2,18))
plot(exp.gn.1, exp.gn.2, col=use.col.2,
  main='non-subset arrays', cex.main=1.5, cex=2, cex.lab=1.5,
  xlab=rf.sig.gn[1], ylab=rf.sig.gn[2], pch=use.pch.2)
legend('bottomright',
  c('B-cell', 'T-cell'), pch=c(1,16), col=c(1,2), cex=1.5)
```

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## RF Variable Selection on ALL (full)



Each condition has a profile / signature (across these genes)

full ALL data: 12,625 genes, 128 arrays

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# RF variable selection with full data set

# set seed and define initial objects
set.seed(123)
eset <- exprs(ALL) # 12625 genes, 128 arrays
cell <- c(rep(0,95),rep(1,33))
# first 95 are B-cell; last 33 are T-cell

print(date())
rf.big <- varSelRF(t(eset),as.factor(cell),
  ntree=10000, ntreeIterat=2000, vars.drop.frac=0.2)
print(date()) # about 9 minutes

rf.gn <- rf.big$selected.vars
# "33039_at" "35016_at" "38319_at"

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# make scatterplot matrix, with points colored by cell type
t.rf <- is.element(rownames(eset),rf.gn)
rf.eset <- t(eset[t.rf,])
# this rf.eset has rows for obs and columns for 3 genes
use.pch <- c(rep(1,95),rep(16,33))
use.col <- cell+1
pairs(rf.eset,col=use.col,pch=use.pch,cex=1.5)
# pairs function makes scatterplot matrix of rf.eset cols.

# Now - make a profile plot (parallel coordinates plot)
library(MASS)
parcoord(rf.eset, col=cell+1, lty=cell+1,
  lwd=3, var.label=TRUE)
legend(1.2,.15,c('B-cell','T-cell'),lty=c(1,2),
  lwd=3,col=c(1,2),bty='n')

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## Summary: RF for gene expression data

- Works well even with: many more variables than observations, many-valued categoricals, extensive missing values, badly unbalanced data
- Low error (comparable w/ boosting and SVM)
- Robustness (even with large “noise” in genes)
- Does not overfit
- Fast, and invariant to monotone transformations of the predictors
- Free! (Fortran code by Breiman and Cutler)
- Returns the importance of predictors (gene)
- Little need to tune parameters
- But, no: statistical inference

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