Statistical methods for bioinformatics
Gene expression: case study (de Vijver et al.)

Cedric Lood

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1 Dataset

The dataset under consideration consists of values of about 5000 gene expressions (obtained through a microarray technology) for 188 patients. Some of the patients developed distant metastases while others did not, and the goal is to see if we can use the information from the levels of gene expressions to predict the metatstases phenotype and subsequently use appropriate treatment for the patient.
One thing to note from the dimensions of the dataset is that we are falling in the case of so-called high dimensionality with a number of observations n that is over an order of magnitude lower than that of the p predictors. Among other things, this prevents the use of least square methods, common in linear models.
One of the risk when trying to come up with a reduced gene panel that would predict the evolution of the cancer is that high levels of colinearity between the predictors likely exists. This renders the set of gene selected contingent on the particular dataset obtained.

2 Predictive potential

Here are the libraries I used throughout the analysis:

```
library(glmnet)
library(polycor)
library(ROCR)
library(leaps)
library(pls)

load("VIJVER.Rdata")
```

As a first approach, I tried to search systematically through the dataset for variables that would correlate with the binary outcome with a correlation score above 0.45 (for a score of 0.5, the procedure returns only 2 results).

```
## Systematic identification of correlated genex/outcome
genes <- character()
i <- 1
for(gene in names(data)[2:length(data)]) {
```

```
    if(abs(hetcor(data$meta, data[gene])$correlations[1,2]) > 0.45){
        genes[i] <- gene
        i <- i + 1
    }
}
```

Here is the list of genes returned:

```
 [1] "NM_000987"      "NM_003258"      "NM_003295"      "NM_004119"
 [5] "NM_004203"      "NM_002808"      "NM_002811"      "NM_012291"
 [9] "NM_013277"      "NM_003981"      "NM_004701"      "M96577"
[13] "NM_007019"      "NM_007057"      "NM_007267"      "NM_007274"
[17] "NM_006607"      "NM_016185"      "Contig48913_RC" "NM_018410"
[21] "NM_001168"      "NM_002106"
```

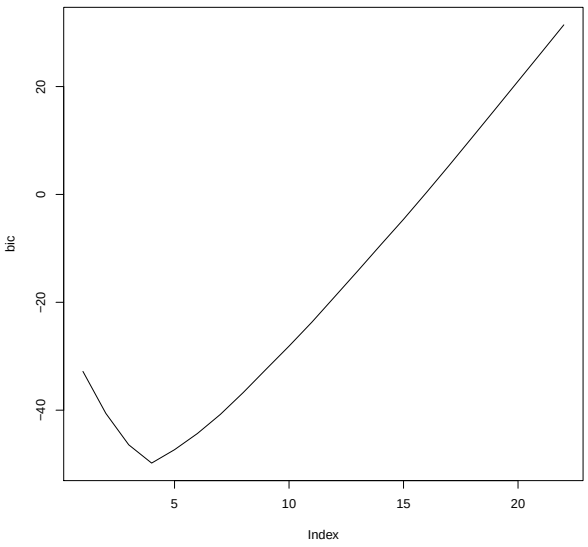
Which I then used to build a logistic model, with a best subset selection approach. For the selection, I used the BIC metric to select the subset of variable and obtained a ROC curve for the classifier obtained (see graphics below).

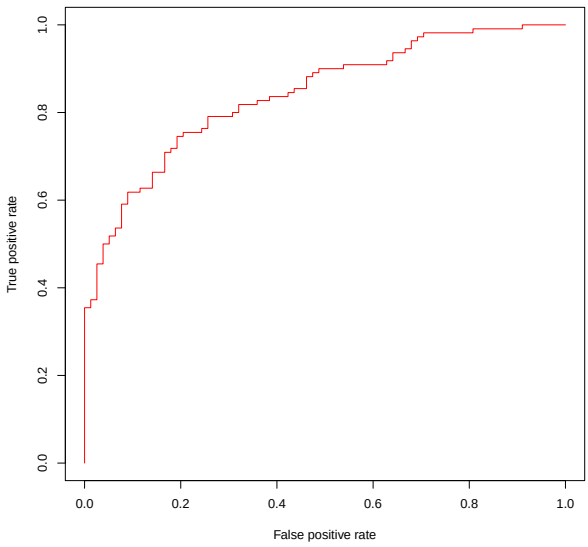
```
## Use the identified variables to create a model
regfit.full <- regsubsets(data.meta~., reduced.data, nvmax = length(genes))
reg.summary <- summary(regfit.full)

## using bic to make a decision, n=4
which.min(reg.summary$bic)
coef(regfit.full,4)

## fitting model with the 4 params
reg <- glm(meta~NM_000987+NM_003258+NM_004119+NM_002811, data=data,family = binomial(link=logi
summary(reg)
reg.probs <- predict(reg, type="response")
contrasts(data$meta)
table(data$meta, fitted(reg)>0.5)
predict <- fitted(reg)
pred <- prediction(predict, data$meta)
perf <- performance(pred, measure="tpr", x.measure = "fpr")
performance(pred, measure="auc")

## plotting the results
pdf("bic-auc.pdf", width = 16, height = 8)
par(mfrow = c(1,2))
plot(reg.summary$bic, ylab="bic", type="l")
plot(perf, col="red")
dev.off()
```



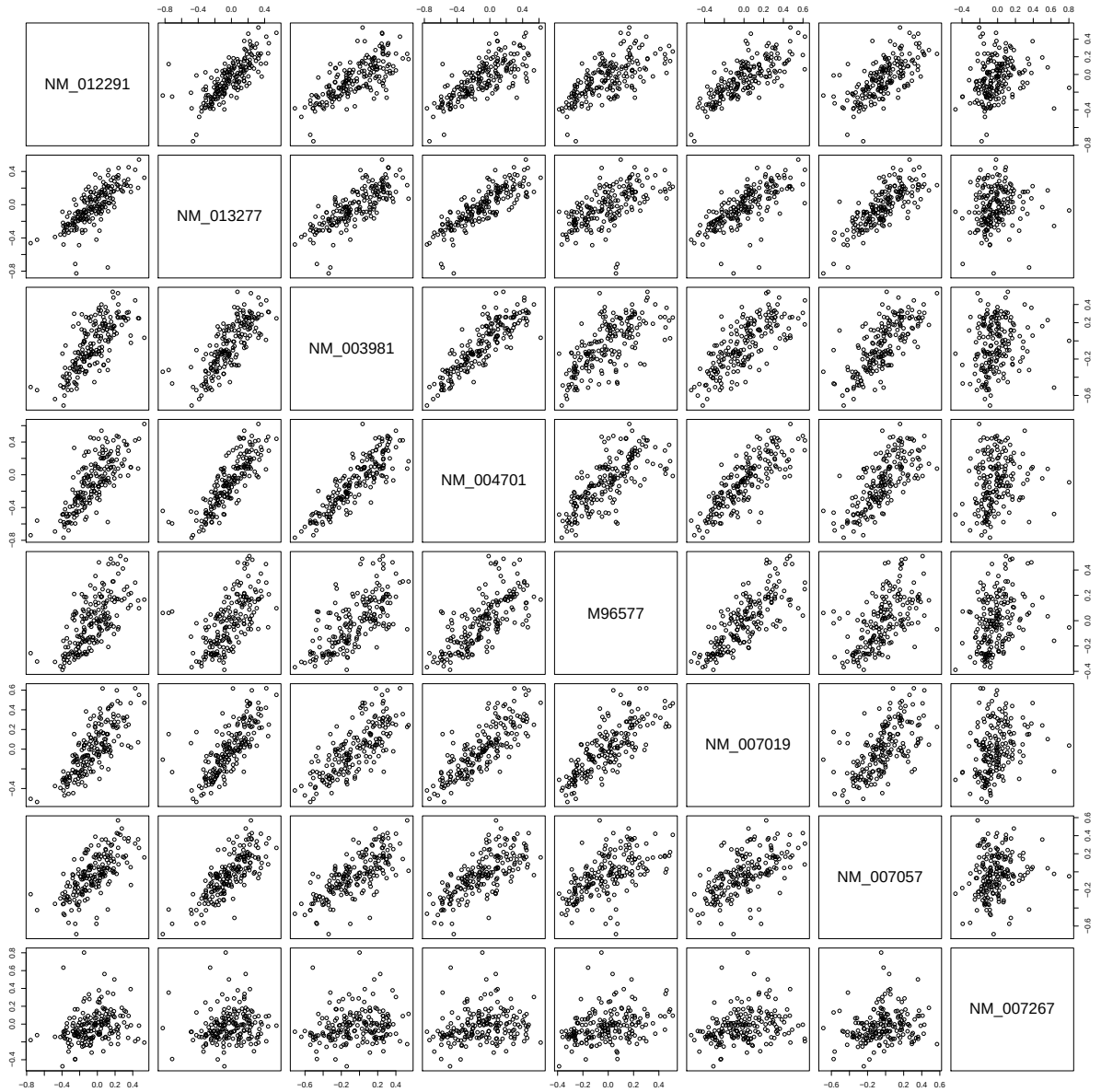


3 Collinearity structure

Given the size and the nature of the data collected (RNA transcript levels), we can certainly expect colinearity between different predictors. One simple justification for it is that often multiple genes encode the information necessary for a given pathway. Those will then be transcribed together in order to enable the different steps in the metabolic pathway to be succesful.
This represents a challenge in the statistical analysis as colinearity leads to unstability in the modelling. Typically, the fitted coefficients of the model will vary significantly with small perturbations in the dataset.
Working with the reduced dataset of 22 transcript levels created above, one can alreay show multiple examples of such colinearity:

```
pairs(reduced.data[,2:8]) # no good examples in this subset (max=0.5)
cor(reduced.data$NM_002808,reduced.data$NM_002811)
pairs(reduced.data[,9:16]) # good amount of corelated pairs (max=0.9)
cor(reduced.data$NM_003981,reduced.data$NM_004701)
```

```
> cor(reduced.data$NM_002808,reduced.data$NM_002811)
[1] 0.5072933
> cor(reduced.data$NM_003981,reduced.data$NM_004701)
[1] 0.8694202
```



4 Phenotype prediction

I used the ridge regression and lasso approaches to build classifiers. Another one was suggested - principal component regression - but it did not work due to the binary nature of the response variable. For both approaches, I used CV to determine the value of the hyperparameter λ

The ridge regression does not perform variable selection, so all the coefficients were present, but for lasso, only 30 predictors were kept in the final model.

Below the source code are some metrics used to measure the quality of the models and graphs of the ROC for the classifiers.

```
set.seed(1)
x <- model.matrix(meta~.,data)[,-1]
```

```
y <- data$meta

## Ridge regression with CV
grid <- 10^seq(10,-2,length=100)
ridge.cv <- cv.glmnet(x,y,alpha=0, family='binomial')
plot(ridge.cv)
ridge.bestlam <- cv.out$lambda.min

ridge.mod <-  glmnet(x,y,alpha=0,lambda=grid,family='binomial')
ridge.pred <- predict(ridge.mod, type="response",s=ridge.bestlam, newx=x)
table(y, ridge.pred>0.5)

ridge.pred <- prediction(ridge.pred, y)
ridge.perf <- performance(ridge.pred, measure="tpr", x.measure = "fpr")
performance(ridge.pred, measure="auc")

## Lasso
lasso.cv <- cv.glmnet(x,y,alpha=1,lambda=grid,family='binomial')
plot(lasso.mod)
lasso.bestlam <- lasso.cv$lambda.min

lasso.mod <- glmnet(x,y,alpha=1,lambda=grid,family='binomial')
lasso.coef <- predict(lasso.mod, type="coefficients",s=lasso.bestlam)
lasso.coef[lasso.coef!=0]
lasso.pred <- predict(lasso.mod, type="response",s=lasso.bestlam, newx=x)
table(y, lasso.pred>0.5)

lasso.pred <- prediction(lasso.pred, y)
lasso.perf <- performance(lasso.pred, measure="tpr", x.measure = "fpr")
performance(lasso.pred, measure="auc")

## plotting results of ridge and lasso (ROC)
pdf("roc-lasso-ridge.pdf", width = 16, height = 8)
par(mfrow = c(1,2))
plot(ridge.perf, col="red", main="ridge regression")
plot(lasso.perf, col="red", main="lasso")
dev.off()

> lasso.coef[lasso.coef!=0]
<sparse>[ <logic> ] : .M.sub.i.logical() maybe inefficient
 [1]  0.247743870  0.011295692  0.346844990 -0.895657534  0.552269072
 [6] -0.423686469  0.786917703 -1.272024734 -0.229754590  0.003260426
[11] -1.290165435 -0.855594832 -0.904164172 -0.182372158  0.073626879
[16]  0.001791241 -0.145971738 -0.532059542 -0.168487071 -0.057471682
[21] -0.069827181 -0.344073193 -0.030836020  0.072757656  0.111413268
[26] -0.081664952 -0.007007882  0.043994563 -0.435761494  0.743092530

> table(y, ridge.pred>0.5)
```

```
y      FALSE TRUE
  DM      61   17
  NODM     6  104
```

```
> performance(ridge.pred, measure="auc")
Slot "y.values":
[[1]]
[1] 0.9424242
```

```
> table(y, lasso.pred>0.5)
```

```
y      FALSE TRUE
  DM      58   20
  NODM     8  102
```

```
> performance(lasso.pred, measure="auc")
Slot "y.values":
[[1]]
[1] 0.9424242
```