University of BRISTOL

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Detection and prediction using DNA methylation, practical

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Getting started

To start, everyone please make sure that:

- your directory is correctly set
- you only have the `dataset.rda` file loaded in active memory

If not, this should get you up to speed:

```
## for example:
setwd("O:/teaching/advancedEpi_shortCourse")
rm(list=ls())
```

```
load("dataset.rda")
```

```r
str(samples)
table(samples$smoking)
```

current	former	never
22	263	179

- Our current smoking variable has 3 categories, but we want to work with a binary outcome
- Let’s do that by collapsing the **former** and **current** subjects into a single category of ever smokers

Let’s add an never/ever smoking variable to our `samples` data frame:

```
samples$never.smoke <- sign(samples$smoking=="never")

## be sure it matches the counts we'd expected
table(samples$never.smoke)
```

```
>
>   0   1
> 285 179
```

- When I talk about predicting smoking going from now on I’ll be referring to this variable

Using a single CpG site as a predictor of smoking

Single CpG predictor

- Let's start by seeing how well just the single top hit CpG does at predicting smoking
  - cg05575921 in the *AHRR* gene
- Start by adding it as a new variable to our `samples` data frame

```
samples$cg05575921 <- meth["cg05575921", ]
```

Single CpG predictor

- We'll use the `pROC` package to see how well `cg05575921` does at predicting `never.smoke`

```r
## load the pROC package
require("pROC")

## use the formula-based syntax of the package
roc.out <- roc(never.smoke ~ cg05575921, data = samples)
```

Single CpG predictor

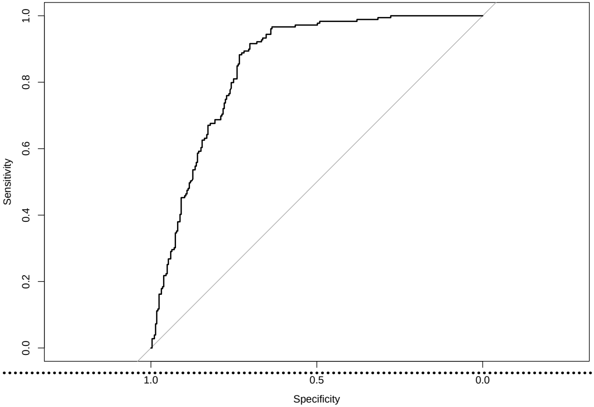
```
## load the pROC package
require("pROC")

## use the formula-based syntax of the package
roc.out <- roc(never.smoke ~ cg05575921, data = samples)
```

```
roc.out # man!!
```

```
>
> Call:
> roc.formula(formula = never.smoke ~ cg05575921, data = samp
>
> Data: cg05575921 in 285 controls (never.smoke 0) < 179 case
> Area under the curve: 0.851
```

Single CpG predictor

```r
plot.roc(roc.out)
```



Using published coefficients to make a prediction score

Methylation score from published coefficients

- Let's compare how well our single site, `cg05575921`, performs at predicting smoking to a smoking score derived from the published coefficients of the largest blood EWAS meta-analysis to date by Joehanes et al. 2016
- The coefficients were reported in their supplemental material
  - I've conveniently read them into R for you so they're ready to use (you're welcome!)
  - Try loading them into R

```r
load("joehanes2016_st2_bonf.rda")
```

Methylation score from published coefficients

- The `joehanes` object has summary information on the 2617 CpGs that were significant at a Bonferroni p-value threshold in the original meta-analysis and that were available in our methylation dataset

```
str(joehanes)
```

```
> 'data.frame': 2617 obs. of  14 variables:
>  $ probe.id            : chr  "cg16145216" "cg19406367" "cg
>  $ infinium.design.type: chr  "I" "II" "II" "II" ...
>  $ chromosome          : chr  "1" "1" "1" "11" ...
>  $ location..hg19.     : chr  "42,385,662" "66,999,929" "2,
>  $ strand              : chr  "R" "R" "F" "R" ...
>  $ gene.symbol         : chr  "HIVEP3" "SGIP1" "SKI" "CUGBP
>  $ effect              : num  0.0298 0.0175 -0.0122 -0.0124
>  $ std..error          : num  0.002 0.0013 0.0009 0.0009 0.
```

Methylation score from published coefficients

- Let's restrict our big methylation data object, `meth`, to just the CpGs that are in the `joehanes` list
- This keeps the CpGs that we expect to be most related to smoking behavior while reducing the size of the data were working with

```
meth <- meth[joehanes$probe.id, ]
```

Methylation score from published coefficients

Transpose the methylation matrix so that the CpGs are the columns of the object, like they would be if they were normal variables

```
X <- t(meth)
```

- This puts our methylation covariates in the matrix-notation that is consistent with most mathematical notation of linear models

Methylation score from published coefficients

That is, while you may be used to thinking of linear regression in this format:

- $E(Y|X) = \beta_0 + \beta_1 X_1 ... \beta_j X_j$

You can also write an equation for the β coefficients in matrix notation as below:

- $\hat{\beta} = (X^T X)^{-1} X^T y$

This is a handy way of describing our coefficients when we're thinking about how to use them to generate fitted/predicted values:

- $\hat{y} = X\hat{\beta}$

Methylation score from published coefficients

Let's also go ahead and make a nice named vector of the `joehanes` coefficients that we can apply to our observed methylation and generate our smoking score ($\hat{y}$):

```
coefs <- joehanes$effect
names(coefs) <- joehanes$probe.id
```

QUESTION TIME!

1. Apply the `joehanes` coefficients to our observed methylation values
2. Add the score to your existing `samples` data frame
3. Calculate the AUC for this predictor
4. Draw the ROC curve
5. Does the `joehanes` score predict never/ever smoking better than just cg05575921 alone?

ANSWER TIME!

1. Apply the `joehanes` coefficients to our observed methylation values

```
y.hat <-   X %*% coefs
```

2. Add the score to your existing `samples` data frame

```
samples$y.hat <- as.vector(y.hat)
```

3. Calculate the AUC for this predictor

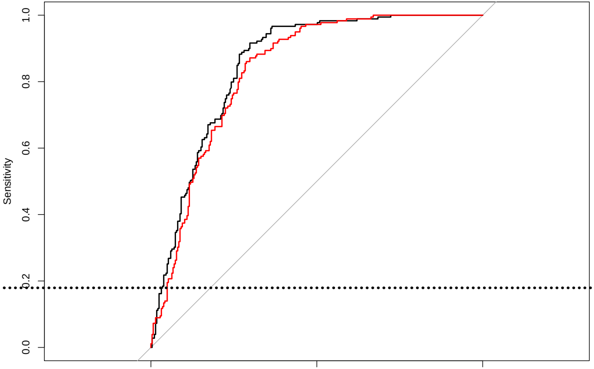
```
roc.out.again <- roc(never.smoke ~ y.hat, data = samples)
roc.out.again$auc
```

```
> Area under the curve: 0.832
```

ANSWER TIME!

4. Draw the ROC curve

```
plot.roc(roc.out)
lines.roc(roc.out.again, col="red")
```



ANSWER TIME!

5. Does the `joehanes` score predict never/ever smoking better than just cg05575921 alone?

ANSWER TIME!

5. Does the `joehanes` score predict never/ever smoking better than just cg05575921 alone?

No! Not according to the AUCs:

```
roc.out$auc
```

```
> Area under the curve: 0.851
```

```
roc.out.again$auc
```

```
> Area under the curve: 0.832
```

Train and test a new predictor with cross validation

Making folds

Let’s chop our dataset up into several subsets so that we can train and test a prediction model on different observations in our dataset

- We could do this using the base function, `sample()`, but if we want to make more than a couple folds this gets messy

We’ll use the `caret` package to automatically partition our data into 10-folds:

```
require(caret)

set.seed(20180314)
Y <- samples$never.smoke
flds <- createFolds(Y, k = 10, list = TRUE,
            returnTrain = FALSE)
```

Making folds

```
str(flds)
```

```
> List of 10
>  $ Fold01: int [1:46] 3 13 20 22 27 36 96 98 111 119 ...
>  $ Fold02: int [1:47] 5 16 17 45 63 77 83 87 92 93 ...
>  $ Fold03: int [1:46] 2 11 15 18 26 32 42 46 65 72 ...
>  $ Fold04: int [1:46] 10 34 47 53 57 61 81 82 85 94 ...
>  $ Fold05: int [1:47] 7 31 40 50 51 54 64 69 70 90 ...
>  $ Fold06: int [1:46] 6 8 9 21 24 33 48 113 121 123 ...
>  $ Fold07: int [1:47] 4 37 39 41 44 67 80 84 89 107 ...
>  $ Fold08: int [1:46] 12 23 25 29 35 49 52 56 59 62 ...
>  $ Fold09: int [1:46] 14 19 28 68 71 79 88 101 106 117 ...
>  $ Fold10: int [1:47] 1 30 38 43 55 58 60 73 75 78 ...
```

- Use the first fold we generated to be our testing dataset
- The rest we'll use for training

```
test <- flds$Fold01
train <- (-test)
```

Training

- Let’s train our own predictor using a lasso model, like we learned about in the lecture earlier
  - E.g. the Liu et al. 2016 DNAm alcohol score used a lasso model

Training

Load the `glmnet` package which has the lasso model function and try fitting on our training data

```r
require(glmnet)
set.seed(420)
fit.lasso <- cv.glmnet(X[train,], Y[train],
    family='binomial', alpha=1, standardize=TRUE,
    type.measure='auc', nfolds = 10)
```

- Remember: lasso picks the shrinkage factor, λ, by internal cross-validation

Lasso coefficients

```r
coef.lasso <- as.matrix(coef(fit.lasso, s = "lambda.min"))
coef.lasso <- data.frame(betas=coef.lasso[,1],
                stringsAsFactors = F)
coef.lasso <- subset(coef.lasso, betas!=0)

str(coef.lasso)
```

```
> 'data.frame': 19 obs. of  1 variable:
>  $ betas: num  -11.964 5.546 1.306 0.378 3.963 ...
```

Lasso coefficients

```
coef.lasso
```

```
>                    betas
> (Intercept) -11.96407920
> cg05951221    5.54642609
> cg05575921    1.30629345
> cg10919522    0.37799224
> cg21566642    3.96254550
> cg25189904    0.94875906
> cg03636183    0.29443121
> cg19859270    7.46925604
> cg06126421    3.62530479
> cg00138101   -3.55277076
> cg24154132   -4.65644918
> cg18956562   -2.14032920
```

Predicting in new data

We can see how well the model we just fit performs in new data by predicting on the observations from the testing set:

```r
pred.lasso <- as.vector(predict(fit.lasso,
    newx = X[test,], type = "response", s = "lambda.min"))
```

ROC

```
roc.out.l <- roc(Y[test], pred.lasso)
roc.out.l$auc
```

```
> Area under the curve: 0.8481
```

- Seems like our performance is quite similar to the other methods we've tried so far

QUESTION TIME!

1. Use another fold of our data to train at least **two** new prediction models for never/ever smoking
   - Any flavor of logistic regression (lm, glm, poisson, interactions, quadratics, any combination of CpGs)
   - Forward/backward stepwise regression
   - Mean value of Y
   - K-nearest neighbors (`class::knn`)
   - Bayes regression (arm::bayesglm)
   - Or any other!
2. For each method, predict the fitted value $\hat{y}$ in a separate fold
3. Calculate the AUCs for each
4. Plot their performance on a ROC curve

ANSWER TIME!

```
X <- as.data.frame(X)
# new folds
test <- flds$Fold02
train <- (-test)

## Model 1: glm using a random subset of cpgs
set.seed(86)
cpgs <- sample(colnames(X), 20) # sample 20 cpgs

fit.glm <- glm(Y[train] ~. ,
    data=X[train,cpgs], family='binomial')

pred.glm <- predict(fit.glm,
    newx = X[test,], type = "response")
```

ANSWER TIME!

```
## Model 2: stepwise glm choosing from 20 random cpgs
fit.step <- step(fit.glm, direction = "backward",
                 trace = 1, k = 2)

pred.step <- predict(fit.step, newdata = X[test,],
                     type = "response")
```

```
> Start:  AIC=492.06
> Y[train] ~ cg00315394 + cg07229212 + cg04017131 + cg2605595
>     cg00756943 + cg16554099 + cg10310310 + cg03222009 + cg2
>     cg21121843 + cg03935116 + cg02363630 + cg04761746 + cg1
>     cg19142553 + cg08202836 + cg17823346 + cg04641860 + cg0
>     cg21329975
>
>              Df Deviance    AIC
```

ANSWER TIME!

```
roc.step <- pROC::roc(Y[test], pred.step)
roc.step$auc
```

```
> Area under the curve: 0.6868
```

ANSWER TIME!

```
## Model 3: knn
fit.knn <- class::knn(train = X[train,], test = X[test,],
    k = 10, cl = Y[train], prob = TRUE)
pred.knn <- (as.numeric(fit.knn) - 1) * attr(fit.knn, "prob")
    (1 - (as.numeric(fit.knn) - 1)) * (1 - attr(fit.knn,
        "prob"))

roc.knn <- pROC::roc(Y[test], pred.knn)
roc.knn$auc
```

```
> Area under the curve: 0.8516
```

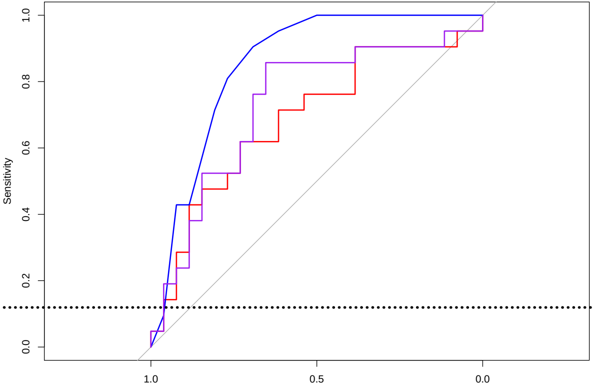
ANSWER TIME!

```
## Model 3: bayes regression
fit.bayes <- arm::bayesglm(Y[train] ~., data = X[train, cpgs]
pred.bayes <- predict(fit.bayes, newdata = X[test,],
                type = "response")

roc.bayes <- pROC::roc(Y[test], pred.bayes)
roc.bayes$auc
```

```
> Area under the curve: 0.7308
```

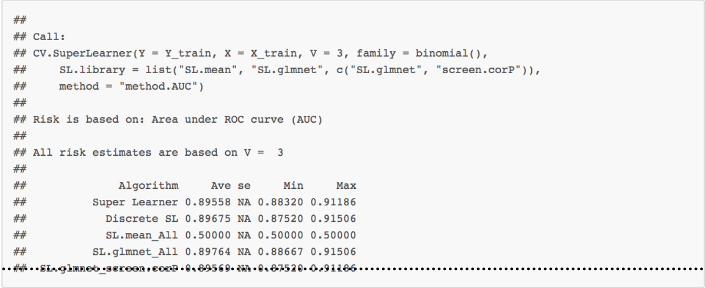
ANSWER TIME!

```
plot.roc(roc.step, col="red")
lines.roc(roc.knn, col="blue")
lines.roc(roc.bayes, col="purple")
```



Next steps

- The SuperLearner package can automate the cross validation using a library of candidate prediction models
  - https://cran.r-project.org/web/packages/SuperLearner/index.html



```
##
## Call:
## CV.SuperLearner(Y = Y_train, X = X_train, V = 3, family = binomial(),
##     SL.library = list("SL.mean", "SL.glmnet", c("SL.glmnet", "screen.corP")),
##     method = "method.AUC")
##
## Risk is based on: Area under ROC curve (AUC)
##
## All risk estimates are based on V =  3
##
##              Algorithm     Ave se     Min     Max
##          Super Learner 0.89558 NA 0.88320 0.91186
##            Discrete SL 0.89675 NA 0.87520 0.91506
##            SL.mean_All 0.50000 NA 0.50000 0.50000
##          SL.glmnet_All 0.89764 NA 0.88667 0.91506
##  SL.glmnet_screen.corP 0.89569 NA 0.87520 0.91186
```