

# Hypoxia Classifier Tutorial

Laura Puente-Santamaria

## Step 1: Load decision trees and gene expression matrix

```
load("Tree_collection.RData")
load("GeneExpression_example.RData")

head(gene_expression)
```

```
##           [,1]
## A1BG       1.000
## A1BG-AS1   5.635
## A1CF       2.000
## A2M       45.972
## A2M-AS1    5.000
## A2ML1      2.243
```

This gene expression matrix is the salmon output corresponding to GSM2390150, an RNA-seq of HUVEC cells grown in hypoxia for 8h.

## Step 2: Ranking percentile.

```
rank_percentile <- matrix( 100*rank( gene_expression ) / length( gene_expression ) )

rownames( rank_percentile ) <- rownames( gene_expression )
colnames( rank_percentile ) <- "Sample 1"

head(rank_percentile)
```

```
##           Sample 1
## A1BG       39.94070
## A1BG-AS1   50.75651
## A1CF       44.06828
## A2M       65.71069
## A2M-AS1   50.03055
## A2ML1      45.44474
```

We rank genes from the least to the most expressed, with 100 being the most expressed gene in the sample, and 0 the least. The trees generated with `rpart` take as input a data frame with a row for samples and columns for variables:

```
rank_percentile <- data.frame( t( rank_percentile ) )

rank_percentile[,1:5]
```

```
##           A1BG A1BG.AS1      A1CF      A2M A2M.AS1
## Sample 1 39.9407 50.75651 44.06828 65.71069 50.03055
```

### Step 3: Classify the sample.

```
decisionTree <- fullTreeCollection[[ 125 ]]  
  
prediction <- predict( decisionTree, rank_percentile )  
  
prediction
```

```
##                N                H  
## Sample 1 0.07352941 0.9264706
```

The resulting matrix, `prediction`, has two columns with the probabilities of the sample to be normoxic (first column) or hypoxic (second column).

### Handling missing data

Decision trees generated with `rpart` require that new data to classify includes all variables used to build the model, in our case, the following 20 genes: ADM, ANKRD37, BHLHE40, BNIP3, BNIP3L, C4orf3, C8orf58, CCNG2, EGLN1, GPR146, KDM3A, MAFF, MIR210HG, NARF, NDRG1, P4HA1, PFKFB3, SLC2A1, TCAF2, and ZNF395.

Occasionally, one or more of these genes will not be present in the expression data for several reasons: a gene might not be included in the reference transcriptome, or those genes not detected in the sample were filtered out. As an example, using a table that is missing data on “ZNF395”, the function `predict` will throw an error:

```
rank_missing <- rank_percentile[ ,  
  c("ADM", "ANKRD37", "BHLHE40", "BNIP3", "BNIP3L", "C4orf3",  
    "C8orf58", "CCNG2", "EGLN1", "GPR146", "KDM3A", "MAFF",  
    "MIR210HG", "NARF", "NDRG1", "P4HA1", "PFKFB3", "SLC2A1", "TCAF2")  
]  
  
predict( decisionTree, rank_missing )
```

```
## Error in eval(predvars, data, env): object 'ZNF395' not found
```

We can solve this issue adding an extra column with NA values to our gene expression table:

```
rank_missing$ZNF395 <- as.numeric( NA )  
  
predict( decisionTree, rank_missing )
```

```
##                N                H  
## Sample 1 0.07352941 0.9264706
```