

DEB RNA no FAIMS S100

Load required packages

```
## corrplot 0.84 loaded
## -- Attaching packages -----
## v ggplot2 3.3.0    v purrr  0.3.4
## v tidyr  1.0.3    v dplyr  0.8.5
## v readr  1.3.1    v forcats 0.5.0
## -- Conflicts -----
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
##
## Attaching package: 'kableExtra'
## The following object is masked from 'package:dplyr':
##
##   group_rows
##
## Attaching package: 'magrittr'
## The following object is masked from 'package:purrr':
##
##   set_names
## The following object is masked from 'package:tidyr':
##
##   extract
##
## Attaching package: 'gridExtra'
## The following object is masked from 'package:dplyr':
##
##   combine
##
## *****
## Note: As of version 1.0.0, cowplot does not change the
##   default ggplot2 theme anymore. To recover the previous
##   behavior, execute:
##   theme_set(theme_cowplot())
## *****
##
## Attaching package: 'cowplot'
```

```
## The following object is masked from 'package:ggpubr':  
##  
##   get_legend  
## Loading required package: lattice  
## Loading required package: survival  
## Loading required package: Formula  
##  
## Attaching package: 'Hmisc'  
## The following objects are masked from 'package:dplyr':  
##  
##   src, summarize  
## The following objects are masked from 'package:base':  
##  
##   format.pval, units  
##  
## Attaching package: 'caret'  
## The following object is masked from 'package:survival':  
##  
##   cluster  
## The following object is masked from 'package:purrr':  
##  
##   lift  
## Loading required package: usethis
```

Loading RNP:XL data into the R environment

I included the manually evaluated datasets for my ASMS talk to see if machine learning can help me with identifying a possible score cutoff or other metric to help reduce the numbers of entries I have to go through after RNP:xl output. The datasets include a merged version of both the S30 and S100 fraction that were initially considered individually:

*Ecoli DEB RNA

Data wrangling

First, we need to add an extra column to each dataset, indicating which cross-linker was used in the experiment.

Then, we need to combine the dataframes.

First filtering step

Remove any entry that does not contain an RNA adduct and fits to a target better than a decoy.

Compute additional metrics (columns)

Peplength

classification of amino acids

Create new column with peptide length and classify identified amino acids in peptides as follows:

1. charged amino acids: R, K, D, E
2. polar amino acids: Q, N, H, S, T, Y, C, W
3. hydrophobic amino acids: A, I, L, M, V, P, G

Peptide pI (isoelectric point)

Compute the isoelectric point for each peptide in each entry.

aI (aliphatic index)

Compute the aliphatic Index for each peptide in each entry. See the following paper for more info:

Thermostability and aliphatic index of globular proteins. J Biochem. 1980 Dec;88(6):1895-8.

“A statistical analysis shows that the aliphatic index, which is defined as the relative volume of a protein occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine), of proteins of thermophilic bacteria is significantly higher than that of ordinary proteins. The index may be regarded as a positive factor for the increase of thermostability of globular proteins.”

In general, an aI of greater 92.6 is particularly stable thermally as found in thermophiles. Now, since we are investigating tryptic peptides, I could only find one paper on that matter.

GETTING INTIMATE WITH TRYPSIN, THE LEADING PROTEASE IN PROTEOMICS Published online 15 June 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mas.21376

According to this paper, human proteins result in 61 tryptic peptides on average.

proposed cross-linking amino acid

Hydrophobicity index

Molecular weight of peptide

Note: returns the average masses, not the monoisotopic masses!

Machine learning

Split dataset

Separate different species of nucleotides (RNA, DNA) and cross-linker (DEB, DEB).

Setting up the dataset

Harmonize the number of trues and falses and dropping all categorical columns. 50% T and 50% F

```
set.seed(42)
drops_general<-c("precursor_purity", "RNPxl_a_ion_score")

ml_DEB_RNA<-data1 %>%
  filter(XLinker == "DEB", Curated == "0", Nucleotides == "RNA") %>%
  sample_n(459) %>%
  rbind(RNA_DEB)
rows_DEB_RNA <- sample(nrow(ml_DEB_RNA))
ml_DEB_RNA <- ml_DEB_RNA[rows_DEB_RNA, ] %>%
  dplyr::select_if(is.numeric)
ml_DEB_RNA<-ml_DEB_RNA[, !(colnames(ml_DEB_RNA) %in% drops_general)]
```

Splitting into train and test data

I will use 80% of the data to train, and 20% to test the model

```
ml_DEB_RNA$Curated<-as.factor(ml_DEB_RNA$Curated)
ml_DEB_RNA$Curated<-factor(ml_DEB_RNA$Curated, levels = c("0", "1"), labels = c("False", "True"))
ml_DEB_RNA_train <- ml_DEB_RNA[1:734, ]
ml_DEB_RNA_test  <- ml_DEB_RNA[735:918, ]
```

KNN

K nearest neighbor classification based on numeric variables, min-max normalized.

```
library(class)
library(gmodels)

knn_drop <- c("Curated", "isotope_error", "RNXPxl_a_ion_score", "RNXPxl_precursor_purity")

normalize <- function(x) {
  return((x-min(x))/(max(x)-min(x)))
}

ml_DEB_RNA_test_knn<-ml_DEB_RNA_test[,!(names(ml_DEB_RNA_test) %in% knn_drop)]
ml_DEB_RNA_test_knn<-as.data.frame(lapply(ml_DEB_RNA_test_knn[1:39], normalize))
ml_DEB_RNA_test_knn_labels<-ml_DEB_RNA_test[1:184, 5]

ml_DEB_RNA_train_knn<-ml_DEB_RNA_train[,!(names(ml_DEB_RNA_train) %in% knn_drop)]
ml_DEB_RNA_train_knn<-as.data.frame(lapply(ml_DEB_RNA_train_knn[1:39], normalize))
ml_DEB_RNA_train_knn_labels<-as.data.frame(ml_DEB_RNA_train[1:734, 5])
```

Evaluate Model Performance DEB RNA

Here, I chose 5 neighbors:

```
DEB_RNA_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = ml_DEB_RNA_test_knn,
  cl = ml_DEB_RNA_train_knn_labels$Curated, k =5 )
CrossTable(x = ml_DEB_RNA_test_knn_labels$Curated, y = DEB_RNA_knn_pred, prop.chisq = F)
```

```
##
##
##   Cell Contents
## |-----|
## |                N |
## |      N / Row Total |
## |      N / Col Total |
## |      N / Table Total |
## |-----|
##
##
## Total Observations in Table:  184
##
##
##                                | DEB_RNA_knn_pred
## ml_DEB_RNA_test_knn_labels$Curated |      False |      True | Row Total |
## -----|-----|-----|-----|
```

```
##           False |         79 |         17 |         96 |
##           |     0.823 |     0.177 |     0.522 |
##           |     0.859 |     0.185 |           |
##           |     0.429 |     0.092 |           |
## -----|-----|-----|-----|
##           True |         13 |         75 |         88 |
##           |     0.148 |     0.852 |     0.478 |
##           |     0.141 |     0.815 |           |
##           |     0.071 |     0.408 |           |
## -----|-----|-----|-----|
##           Column Total |         92 |         92 |         184 |
##           |     0.500 |     0.500 |           |
## -----|-----|-----|-----|
##
##
```

Decision trees and classification rules

C5.0

```
library(C50)

C5_drop <- c("isotope_error", "RNPx1_a_ion_score", "precursor_purity")

ml_DEB_RNA_train_C5<-ml_DEB_RNA_train[,!(names(ml_DEB_RNA_train) %in% C5_drop)]
ml_DEB_RNA_test_C5<-ml_DEB_RNA_test[,!(names(ml_DEB_RNA_test) %in% C5_drop)]
```

DEB RNA

with boosting and penalty for false positives

penalize False Positives 4 times more than false negatives.

```
matrix_dimensions<- list(c("True", "False"), c("True", "False"))
names(matrix_dimensions) <- c("predicted", "actual")
error_cost <- matrix(c(0,1,4,0), nrow = 2, dimnames = matrix_dimensions)

C5_model_DEB_RNA_boost_pen<-C5.0(x=ml_DEB_RNA_train_C5[-5], y=ml_DEB_RNA_train_C5$Curated,
                                costs = error_cost, trials = 10)
summary(C5_model_DEB_RNA_boost_pen)
```

```
##
## Call:
## C5.0.default(x = ml_DEB_RNA_train_C5[-5], y =
## ml_DEB_RNA_train_C5$Curated, trials = 10, costs = error_cost)
##
##
## C5.0 [Release 2.07 GPL Edition]      Wed Jun 23 11:29:39 2021
## -----
##
## Class specified by attribute `outcome'
##
## Read 734 cases (40 attributes) from undefined.data
## Read misclassification costs from undefined.costs
```

```

##
## ----- Trial 0: -----
##
## Decision tree:
##
## RNPxl_total_MIC > 0.2365065:
## :...aa_class_polar > 4:
## :   :...RNPxl_pl_im_MIC <= 0.01192007: True (5)
## :   :   RNPxl_pl_im_MIC > 0.01192007: False (4/1)
## :   aa_class_polar <= 4:
## :   :...Hydro <= 0.6: True (205/3)
## :   :   Hydro > 0.6:
## :   :   :...pI <= 9.45: False (6/3)
## :   :   :   pI > 9.45: True (8)
## RNPxl_total_MIC <= 0.2365065:
## :...aa_class_hydrophobic > 3:
## :   :...RNPxl_pl_pc_MIC <= 0: False (322/26)
## :   :   RNPxl_pl_pc_MIC > 0:
## :   :   :...RNPxl_pl_pc_MIC > 0.04382946: True (8)
## :   :   :   RNPxl_pl_pc_MIC <= 0.04382946:
## :   :   :   :...RNPxl_modds <= 0.3741593: False (25/3)
## :   :   :   :   RNPxl_modds > 0.3741593:
## :   :   :   :   :...RNPxl_RNA_MASS_z0 <= 449.0948: False (5)
## :   :   :   :   :   RNPxl_RNA_MASS_z0 > 449.0948: True (14)
## aa_class_hydrophobic <= 3:
## :...aa_class_charged <= 2:
## :   :...RNPxl_pl_modds <= 0.4927427: False (14/9)
## :   :   RNPxl_pl_modds > 0.4927427:
## :   :   :...RNPxl_precursor_score <= 0.001193983: True (60)
## :   :   :   RNPxl_precursor_score > 0.001193983: False (1)
## aa_class_charged > 2:
## :...RT <= 818.5449: True (7)
## :   RT > 818.5449:
## :   :...RNPxl_pl_modds <= 0.6882493: False (19/2)
## :   :   RNPxl_pl_modds > 0.6882493:
## :   :   :...pI <= 4.43: True (10)
## :   :   :   pI > 4.43:
## :   :   :   :...RNPxl_pl_err <= 0.006021813: False (8/1)
## :   :   :   :   RNPxl_pl_err > 0.006021813:
## :   :   :   :   :...RNPxl_immonium_score <= 0.02881235: True (12)
## :   :   :   :   :   RNPxl_immonium_score > 0.02881235: False (1)
##
## ----- Trial 1: -----
##
## Decision tree:
##
## mw > 1856:
## :...RNPxl_pl_modds <= 0.8764216: False (128.9/3)
## :   RNPxl_pl_modds > 0.8764216:
## :   :...RNPxl_err <= 0.002779919: True (40.4/0.8)
## :   :   RNPxl_err > 0.002779919: False (45.6/22.1)
## mw <= 1856:
## :...RNPxl_pl_modds <= 0.04243729: False (18.2/2.3)
## :   RNPxl_pl_modds > 0.04243729:

```

```

##      :...RNPxl_err > 0.009156602: True (73.1/0.8)
##      RNPxl_err <= 0.009156602:
##      :...precursor > 973.7704: False (19/3)
##      precursor <= 973.7704:
##      :...RNPxl_total_MIC > 0.3725607: True (79.6)
##      RNPxl_total_MIC <= 0.3725607:
##      :...aa_class_hydrophobic > 7: False (31.1/14.4)
##      aa_class_hydrophobic <= 7:
##      :...pI > 11.13: False (16.7/5.3)
##      pI <= 11.13:
##      :...RNPxl_immonium_score > 0.008700988:
##      :...Peplength <= 7: True (51.7/3)
##      :   Peplength > 7:
##      :   :...RNPxl_marker_ions_score > 0.0006902145: True (22.8/1.5)
##      :   :   RNPxl_marker_ions_score <= 0.0006902145: [S1]
##      RNPxl_immonium_score <= 0.008700988:
##      :...RNPxl_partial_loss_score > 6.786638: True (100.5)
##      RNPxl_partial_loss_score <= 6.786638:
##      :...Peplength > 10: False (12.1/1.5)
##      Peplength <= 10:
##      :...aIndex > 128.12: False (5.3/0.8)
##      aIndex <= 128.12:
##      :...Peplength > 9: True (69.2)
##      Peplength <= 9:
##      :...pI <= 4.18: False (8.3/3.8)
##      pI > 4.18: [S2]
##
## SubTree [S1]
##
## RNPxl_partial_loss_score > 13.77283: True (9.9)
## RNPxl_partial_loss_score <= 13.77283:
## :...mw <= 861.97: True (9.9)
##   mw > 861.97:
##   :...RNPxl_pl_MIC <= 0.05950294: False (49.7/13.6)
##   RNPxl_pl_MIC > 0.05950294: True (15.2/1.5)
##
## SubTree [S2]
##
## RNPxl_Da_difference <= -0.0001694992: False (34.4/26.6)
## RNPxl_Da_difference > -0.0001694992: True (133.8/2.3)
##
## ----- Trial 2: -----
##
## Decision tree:
##
## mw > 2184.58: False (106.7/20.2)
## mw <= 2184.58:
## :...RNPxl_total_MIC > 0.2095862: True (384.2/11.5)
##   RNPxl_total_MIC <= 0.2095862:
##   :...score <= 0.0594036: True (70.1/0.6)
##   score > 0.0594036:
##   :...RNPxl_pl_modds <= 0.8817645:
##     :...RNPxl_precursor_score > 0.0002412407: True (30.2)
##     :   RNPxl_precursor_score <= 0.0002412407:

```

```

##           :   ...Peplength > 12: False (64.6/5)
##           :       Peplength <= 12:
##           :           ...RNPxl_Da_difference <= -0.0001225632: False (21.8/2.9)
##           :               RNPxl_Da_difference > -0.0001225632:
##           :                   ...RNPxl_err <= 0.004044351: False (62.2/43.4)
##           :                       RNPxl_err > 0.004044351: True (46/0.6)
##           RNPxl_pl_modds > 0.8817645:
##           :   ...Hydro > 0.28: False (8.2/0.6)
##           :       Hydro <= 0.28:
##           :           ...RNPxl_immonium_score <= 0.008185891:
##           :               ...RNPxl_total_MIC <= 0.06030513: False (19.3/15.2)
##           :                   : RNPxl_total_MIC > 0.06030513: True (199/2.3)
##           :                       RNPxl_immonium_score > 0.008185891:
##           :                           ...RNPxl_pl_err <= 0.003974453: False (10.1)
##           :                               RNPxl_pl_err > 0.003974453:
##           :                                   ...aa_class_polar <= 5: True (95.1/8.2)
##           :                                       aa_class_polar > 5: False (4.7)
##
## ----- Trial 3: -----
##
## Decision tree:
##
## aa_class_hydrophobic <= 3:
## :...RNPxl_RNA_MASS_z0 <= 391.0781: False (31.7/20)
## :   RNPxl_RNA_MASS_z0 > 391.0781:
## :       ...Hydro <= -2.13: False (32.3/24.7)
## :           Hydro > -2.13: True (395.6/9.2)
## aa_class_hydrophobic > 3:
## :...RNPxl_total_MIC > 0.3001701:
## :   ...RNPxl_mass_error_p <= 1.941169: True (126.3)
## :       : RNPxl_mass_error_p > 1.941169: False (7.7/3.2)
## :           RNPxl_total_MIC <= 0.3001701:
## :               ...mw > 2405.7: False (50.9)
## :                   mw <= 2405.7:
## :                       ...RNPxl_pl_modds <= 0.07640828: False (21.3)
## :                           RNPxl_pl_modds > 0.07640828:
## :                               ...RNPxl_immonium_score > 0.008149372:
## :                                   ...RNPxl_mass_error_p <= 1.912772: False (89.1/21)
## :                                       : RNPxl_mass_error_p > 1.912772: True (81.1/12.7)
## :                                           RNPxl_immonium_score <= 0.008149372:
## :                                               ...RNPxl_err <= 0.001154643: False (19.5/6.8)
## :                                                   RNPxl_err > 0.001154643:
## :                                                       ...score <= 0.813925: True (207.1/8.2)
## :                                                           score > 0.813925:
## :                                                               ...RNPxl_pl_Morph <= 8.029901: False (18)
## :                                                                   RNPxl_pl_Morph > 8.029901: True (49.3/5.9)
##
## ----- Trial 4: -----
##
## Decision tree:
##
## RNPxl_immonium_score > 0.02881235:
## :...RNPxl_pl_pc_MIC <= 0.01601188: False (45/1.1)
## :   RNPxl_pl_pc_MIC > 0.01601188: True (9.6)

```



```

## RNPxl_immonium_score <= 0.02881235:
## :...RNPxl_total_MIC > 0.2148431:
##   :...aa_class_polar <= 6: True (325/12.5)
##   :   aa_class_polar > 6: False (8/6.1)
## RNPxl_total_MIC <= 0.2148431:
##   :...aa_class_hydrophobic <= 3:
##   :   :...precursor <= 524.2557: False (8.2/3.7)
##   :   :   precursor > 524.2557:
##   :   :     :...RNPxl_immonium_score > 0.009534214: False (64.4/50)
##   :   :     :   RNPxl_immonium_score <= 0.009534214:
##   :   :     :     :...RNPxl_score <= 0.8732423: True (253.2/6.8)
##   :   :     :     :   RNPxl_score > 0.8732423: False (3.8)
##   aa_class_hydrophobic > 3:
##   :...aa_class_hydrophobic > 12: False (22.1)
##   :   aa_class_hydrophobic <= 12:
##   :     :...RNPxl_pl_pc_MIC > 0.04382946: True (35.6)
##   :     :   RNPxl_pl_pc_MIC <= 0.04382946:
##   :     :     :...RNPxl_pl_Morph > 12.05118: True (50.6/3.8)
##   :     :     :   RNPxl_pl_Morph <= 12.05118:
##   :     :     :     :...aa_class_charged > 5: False (24.5)
##   :     :     :     :   aa_class_charged <= 5:
##   :     :     :     :     :...aa_class_polar > 5: False (18.3)
##   :     :     :     :     :   aa_class_polar <= 5:
##   :     :     :     :     :     :...precursor <= 727.8291: False (28.6/9.3)
##   :     :     :     :     :     :   precursor > 727.8291:
##   :     :     :     :     :     :     :...aa_class_charged <= 1: True (25.1/0.7)
##   :     :     :     :     :     :     :   aa_class_charged > 1:
##   :     :     :     :     :     :     :     :...pI > 6.45: False (55.9/18.6)
##   :     :     :     :     :     :     :     :   pI <= 6.45:
##   :     :     :     :     :     :     :     :     :...RNPxl_pl_Morph <= 4.00529: False (8.3)
##   :     :     :     :     :     :     :     :     :   RNPxl_pl_Morph > 4.00529: [S1]
##   :     :     :     :     :     :     :     :     :     :
##   :     :     :     :     :     :     :     :     :     :
## SubTree [S1]
##   :
## RNPxl_pl_im_MIC <= 0.01466738: True (120.9/8.8)
## RNPxl_pl_im_MIC > 0.01466738: False (6.1/0.4)
##   :
## ----- Trial 5: -----
##   :
## Decision tree:
##   :
## aa_class_hydrophobic > 5:
## :...RNPxl_pl_pc_MIC > 0.01601188: True (40.4/1.3)
## :   RNPxl_pl_pc_MIC <= 0.01601188:
## :     :...score <= 0.145311: True (31.8/3.1)
## :     :   score > 0.145311:
## :     :     :...RNPxl_pl_MIC > 0.1462661: True (30.6/1.8)
## :     :     :   RNPxl_pl_MIC <= 0.1462661:
## :     :     :     :...RNPxl_pl_modds <= 1.37235: False (144/33.1)
## :     :     :     :   RNPxl_pl_modds > 1.37235:
## :     :     :     :     :...RT <= 1838.091: True (32.7)
## :     :     :     :     :   RT > 1838.091: False (65.9/30)
## aa_class_hydrophobic <= 5:
## :...RNPxl_total_MIC <= 0.04410198: False (54/33.5)

```

```

## RNPxl_total_MIC > 0.04410198:
## :...aIndex > 78.5:
## :...RNPxl_total_MIC > 0.3017294: True (34.4)
## : RNPxl_total_MIC <= 0.3017294:
## : :...RNPxl_marker_ions_score <= 0.001819908: False (55.7/31.2)
## : RNPxl_marker_ions_score > 0.001819908: True (10.1)
## aIndex <= 78.5:
## :...Peplength <= 6: True (92)
## Peplength > 6:
## :...aa_class_hydrophobic <= 1: False (26.6/18)
## aa_class_hydrophobic > 1:
## :...RNPxl_Morph <= 4.025711: True (206.3/1.1)
## RNPxl_Morph > 4.025711:
## :...precursor_intensity <= 1252038: False (70.7/51)
## precursor_intensity > 1252038:
## :...RNPxl_err <= 0.001018666: False (12.5/6.4)
## RNPxl_err > 0.001018666: True (204.2/3.4)
##
## ----- Trial 6: -----
##
## Decision tree:
##
## Peplength > 10:
## :...RNPxl_total_MIC > 0.2480816: True (68.5/2.2)
## : RNPxl_total_MIC <= 0.2480816:
## : :...aIndex <= 20: True (38.7/3.1)
## : aIndex > 20:
## : :...RNPxl_pl_modds <= 0.8905877: False (110.2/15.7)
## : RNPxl_pl_modds > 0.8905877:
## : :...precursor_error_ppm > 4.391985: True (29.9)
## : precursor_error_ppm <= 4.391985:
## : :...RNPxl_pl_pc_MIC > 0.01709038: True (17)
## : RNPxl_pl_pc_MIC <= 0.01709038:
## : :...RNPxl_immonium_score > 0.008237569: False (29.4/2.9)
## : RNPxl_immonium_score <= 0.008237569:
## : :...RNPxl_mass_error_p <= 1.497245: False (8.7)
## : RNPxl_mass_error_p > 1.497245: True (81.4/5.7)
## Peplength <= 10:
## :...score <= 0.0752907: True (87.8/0.2)
## score > 0.0752907:
## :...aIndex > 86.25:
## :...RNPxl_pl_Morph <= 2.227052: False (9.9)
## : RNPxl_pl_Morph > 2.227052:
## : :...RNPxl_Morph <= 4.530347: True (102.8/11)
## : RNPxl_Morph > 4.530347: False (9.7/2.1)
## aIndex <= 86.25:
## :...RNPxl_RNA_MASS_z0 > 758.1211: False (26.4/18.5)
## RNPxl_RNA_MASS_z0 <= 758.1211:
## :...aa_class_charged <= 2: True (335.1/9.3)
## aa_class_charged > 2:
## :...precursor_error_ppm <= 0.6376348: True (82.6)
## precursor_error_ppm > 0.6376348:
## :...RNPxl_precursor_score > 0.0007802289: False (3.9/0.4)
## RNPxl_precursor_score <= 0.0007802289:

```

```

##           :...RNPxl_pl_err > 0.01726561: False (3.1)
##           RNPxl_pl_err <= 0.01726561:
##           :...RNPxl_marker_ions_score > 0.001386256: False (2.6)
##           RNPxl_marker_ions_score <= 0.001386256:
##           :...RNPxl_pl_modds > 3.219504: False (3.4)
##           RNPxl_pl_modds <= 3.219504:
##           :...mw <= 1219.46: True (144.4/6.9)
##           mw > 1219.46: False (2.5)
##
## ----- Trial 7: -----
##
## Decision tree:
##
## aa_class_hydrophobic > 6:
## :...RNPxl_pl_pc_MIC > 0.01601188: True (30.1/1.7)
## :   RNPxl_pl_pc_MIC <= 0.01601188:
## :     :...OMS_precursor_mz_error_ppm <= 3.760296: False (188.2/80.2)
## :     :   OMS_precursor_mz_error_ppm > 3.760296: True (27.7/1.6)
## aa_class_hydrophobic <= 6:
## :...RNPxl_pl_MIC > 0.1492823: True (76.8)
## :   RNPxl_pl_MIC <= 0.1492823:
## :     :...RNPxl_RNA_MASS_z0 <= 335.123:
## :     :   :...score > 0.929751: True (22.6)
## :     :   :   score <= 0.929751:
## :     :     :...precursor_error_ppm <= 0.5690612: True (17.5/0.2)
## :     :     :   precursor_error_ppm > 0.5690612:
## :     :     :     :...RNPxl_Da_difference > 0.00196853: True (14.2/0.3)
## :     :     :     :   RNPxl_Da_difference <= 0.00196853:
## :     :     :     :     :...RNPxl_peptide_mass_z0 <= 827.5593: True (12.2/0.2)
## :     :     :     :     :   RNPxl_peptide_mass_z0 > 827.5593: False (37.5/0.3)
## :     :     :   RNPxl_RNA_MASS_z0 > 335.123:
## :     :     :     :...RNPxl_total_MIC <= 0.037681: False (41.6/21.6)
## :     :     :     :   RNPxl_total_MIC > 0.037681:
## :     :     :     :     :...aa_class_hydrophobic > 5: False (27.9/15.8)
## :     :     :     :     :   aa_class_hydrophobic <= 5:
## :     :     :     :     :     :...Peplength > 12: False (25.4/16.1)
## :     :     :     :     :     :   Peplength <= 12:
## :     :     :     :     :     :     :...OMS_precursor_mz_error_ppm > 2.227397: True (115/0.2)
## :     :     :     :     :     :     :   OMS_precursor_mz_error_ppm <= 2.227397:
## :     :     :     :     :     :     :     :...RNPxl_Da_difference <= -0.0007595894:
## :     :     :     :     :     :     :     :   :...precursor_error_ppm <= 3.304309: False (55.9/33.1)
## :     :     :     :     :     :     :     :   :   precursor_error_ppm > 3.304309: True (11.9)
## :     :     :     :     :     :     :     :   RNPxl_Da_difference > -0.0007595894:
## :     :     :     :     :     :     :     :     :...RNPxl_modds > 1.71208: False (17.2/12.3)
## :     :     :     :     :     :     :     :     :   RNPxl_modds <= 1.71208:
## :     :     :     :     :     :     :     :     :     :...RT <= 4286.273: True (391.1/13.1)
## :     :     :     :     :     :     :     :     :     :   RT > 4286.273: False (5.1/1.5)
##
## ----- Trial 8: -----
##
## Decision tree:
##
## RNPxl_MIC > 0.3118303: True (111.2)
## RNPxl_MIC <= 0.3118303:

```

```

## :...mw > 2184.58: False (73.9/29.7)
##   mw <= 2184.58:
##     :...RT <= 1262.902: True (274.2/14.8)
##       RT > 1262.902:
##         :...RNPxl_pl_modds <= 0.1330409: False (22)
##           RNPxl_pl_modds > 0.1330409:
##             :...RNPxl_RNA_MASS_z0 <= 351.1179:
##               :...RNPxl_pl_MIC > 0.08438432: True (39.5/1.2)
##                 :   RNPxl_pl_MIC <= 0.08438432:
##                   :     :...RNPxl_pl_modds <= 3.201042: False (68.3/24.2)
##                     :       RNPxl_pl_modds > 3.201042: True (10.7)
##                   RNPxl_RNA_MASS_z0 > 351.1179:
##                     :...pI > 9.3:
##                       :...precursor_intensity <= 953246.9: True (66.8/4)
##                         :   precursor_intensity > 953246.9:
##                           :     :...RNPxl_MIC <= 0.2795199: False (84.3/40.6)
##                             :       RNPxl_MIC > 0.2795199: True (18.6)
##                           pI <= 9.3:
##                             :...Hydro <= -1.89: False (7.5)
##                               Hydro > -1.89:
##                                 :...aa_class_polar <= 1: False (29.3/21.2)
##                                   aa_class_polar > 1:
##                                     :...Peplength <= 9: True (205.9/0.1)
##                                       Peplength > 9:
##                                         :...precursor_error_ppm > 2.518739: False (5.9/0.4)
##                                           precursor_error_ppm <= 2.518739:
##                                             :...pI <= 3.88: False (3.1)
##                                               pI > 3.88: True (170.5/12)
##                                             ##
##                                           ##
##                                         ##
##                                       ##
##                                     ##
##                                   ##
##                                 ##
##                               ##
##                             ##
##                           ##
##                         ##
##                       ##
##                     ##
##                   ##
##                 ##
##               ##
##             ##
##           ##
##         ##
##       ##
##     ##
##   ##
## ## ----- Trial 9: -----
## ##
## ## Decision tree:
## ##
## ## RNPxl_total_MIC <= 0.2148431:
## ## :...aa_class_hydrophobic > 3:
## ## :   :...Peplength > 20: False (27.5)
## ## :   :   Peplength <= 20:
## ## :   :   :...RNPxl_score <= -0.640421: False (145.5/61.1)
## ## :   :   :   RNPxl_score > -0.640421:
## ## :   :   :   :...RNPxl_pl_pc_MIC > 0.0002567632: True (93.8/4.2)
## ## :   :   :   :   RNPxl_pl_pc_MIC <= 0.0002567632:
## ## :   :   :   :   :...RNPxl_immonium_score <= 0.008149372: True (112.5/9.9)
## ## :   :   :   :   :   RNPxl_immonium_score > 0.008149372: False (51.5/12.5)
## ## :   aa_class_hydrophobic <= 3:
## ## :   :...aa_class_charged <= 2: True (179.1/6)
## ## :   :   aa_class_charged > 2:
## ## :   :   :...RNPxl_pl_modds > 2.211792: True (27.6)
## ## :   :   :   RNPxl_pl_modds <= 2.211792:
## ## :   :   :   :...precursor_error_ppm <= 0.51688: True (48.3/3.4)
## ## :   :   :   :   precursor_error_ppm > 0.51688:
## ## :   :   :   :   :...RNPxl_peptide_mass_z0 <= 678.4429: True (7.7)
## ## :   :   :   :   :   RNPxl_peptide_mass_z0 > 678.4429:
## ## :   :   :   :   :   :...RNPxl_Morph <= 2.469005: True (11.3/0.6)
## ## :   :   :   :   :   :   RNPxl_Morph > 2.469005: False (37.1/8.2)

```

```

## RNPxl_total_MIC > 0.2148431:
## :...aa_class_polar > 6: False (13.2/9.6)
##   aa_class_polar <= 6:
##     :...RNPxl_total_MIC > 0.3725607: True (150.8)
##       RNPxl_total_MIC <= 0.3725607:
##         :...RNPxl_score <= -0.9358985: True (99.4)
##           RNPxl_score > -0.9358985:
##             :...Hydro <= -2.04: False (2.8)
##               Hydro > -2.04:
##                 :...RT > 2551.2: False (20/12.4)
##                   RT <= 2551.2:
##                     :...RNPxl_pl_pc_MIC <= 0.1841059: True (119.7/2.3)
##                       RNPxl_pl_pc_MIC > 0.1841059: False (3.1)
##
##
## Evaluation on training data (734 cases):
##
## Trial          Decision Tree
## -----
##   Size      Errors  Cost
##
##   0      19  48( 6.5%)  0.08
##   1      22  92(12.5%)  0.18
##   2      14  62( 8.4%)  0.21
##   3      13 107(14.6%)  0.39
##   4      19  74(10.1%)  0.26
##   5      16  87(11.9%)  0.20
##   6      21  63( 8.6%)  0.27
##   7      18  90(12.3%)  0.22
##   8      16  86(11.7%)  0.32
##   9      18  58( 7.9%)  0.21
## boost                2( 0.3%)  0.00  <<
##
##
##   (a)  (b)  <-classified as
##   ----  ----
##   363      (a): class False
##     2  369  (b): class True
##
##
## Attribute usage:
##
## 100.00% RNPxl_MIC
## 100.00% RNPxl_immonium_score
## 100.00% RNPxl_pl_modds
## 100.00% RNPxl_total_MIC
## 100.00% Peplength
## 100.00% aa_class_hydrophobic
## 100.00% mw
##  98.23% score
##  97.14% RNPxl_pl_MIC
##  96.73% aIndex
##  76.29% RNPxl_err
##  76.16% OMS_precursor_mz_error_ppm

```

```

## 74.52% RT
## 72.21% RNPxl_RNA_MASS_z0
## 70.98% precursor
## 70.30% aa_class_polar
## 65.94% aa_class_charged
## 64.31% RNPxl_pl_pc_MIC
## 61.72% Hydro
## 61.44% RNPxl_score
## 59.54% pI
## 43.32% RNPxl_Da_difference
## 41.14% RNPxl_precursor_score
## 40.87% RNPxl_Morph
## 40.19% precursor_error_ppm
## 38.69% RNPxl_pl_Morph
## 36.51% RNPxl_partial_loss_score
## 35.56% RNPxl_mass_error_p
## 28.47% RNPxl_modds
## 27.52% precursor_intensity
## 24.39% RNPxl_marker_ions_score
## 19.21% RNPxl_pl_err
## 8.31% RNPxl_peptide_mass_z0
## 7.08% RNPxl_pl_im_MIC
##
##
## Time: 0.2 secs

```

```

C5_DEB_RNA_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_test_C5)
CrossTable(ml_DEB_RNA_test_C5$Curated, C5_DEB_RNA_predict_boost_pen, prop.chisq = FALSE,
            prop.c = FALSE, prop.r = FALSE, dnn = c("actual TRUE", "predicted TRUE"))

```

```

##
##
## Cell Contents
## |-----|
## |                N |
## |      N / Table Total |
## |-----|
##
##
## Total Observations in Table: 184
##
##
##      | predicted TRUE
## actual TRUE |      False |      True | Row Total |
## -----|-----|-----|-----|
##      False |      86 |      10 |      96 |
##            |      0.467 |      0.054 |      |
## -----|-----|-----|-----|
##      True |      5 |      83 |      88 |
##           |      0.027 |      0.451 |      |
## -----|-----|-----|-----|
## Column Total |      91 |      93 |      184 |
## -----|-----|-----|-----|
##
##

```

Classifiers

DEB RNA

RIPPER

```
library(rJava)
library(RWeka)
```

```
RIPPER_model_DEB_RNA <- JRip(Curated ~ ., data = ml_DEB_RNA_train_C5)
RIPPER_model_DEB_RNA
```

```
## JRIP rules:
```

```
## =====
```

```
##
```

```
## (mw >= 1164.34) and (RNPxl_total_MIC <= 0.148872) and (RNPxl_pl_modds <= 0.848058) and (score >= 0.4
```

```
## (RNPxl_total_MIC <= 0.236507) and (Hydro >= -0.67) and (RNPxl_immonium_score >= 0.008186) => Curated
```

```
## (RNPxl_peptide_mass_z0 >= 1130.473637) and (RNPxl_total_MIC <= 0.193156) and (RNPxl_RNA_MASS_z0 <= 6
```

```
## (RNPxl_total_MIC <= 0.213968) and (precursor_intensity <= 1138125.875) and (RNPxl_pl_modds <= 0.6873
```

```
## (RNPxl_total_MIC <= 0.28572) and (aa_class_hydrophobic >= 4) and (OMS_precursor_mz_error_ppm >= -0.1
```

```
## (aIndex >= 70) and (RNPxl_total_MIC <= 0.213968) and (RNPxl_pl_MIC <= 0.01229) => Curated=False (12.
```

```
## => Curated=True (364.0/21.0)
```

```
##
```

```
## Number of Rules : 7
```

```
summary(RIPPER_model_DEB_RNA)
```

```
##
```

```
## === Summary ===
```

```
##
```

```
## Correctly Classified Instances          685           93.3243 %
```

```
## Incorrectly Classified Instances         49           6.6757 %
```

```
## Kappa statistic                        0.8665
```

```
## Mean absolute error                    0.1161
```

```
## Root mean squared error                0.2409
```

```
## Relative absolute error                23.2226 %
```

```
## Root relative squared error            48.1898 %
```

```
## Total Number of Instances              734
```

```
##
```

```
## === Confusion Matrix ===
```

```
##
```

```
##   a   b   <-- classified as
```

```
## 342  21 |   a = False
```

```
##  28 343 |   b = True
```

```
RIPPER_DEB_RNA_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_test_C5)
```

```
CrossTable(ml_DEB_RNA_test_C5$Curated, RIPPER_DEB_RNA_predict, prop.chisq = FALSE,
  prop.c = FALSE, prop.r = FALSE, dnn = c("actual TRUE", "predicted TRUE"))
```

```
##
```

```
##
```

```
##   Cell Contents
```

```
## |-----|
```

```
## |                                     N |
```

```
## |           N / Table Total |
```

```
## |-----|
```

```
##
##
## Total Observations in Table: 184
##
##
##      | predicted TRUE
## actual TRUE |      False |      True | Row Total |
## -----|-----|-----|-----|
##      False |      81 |      15 |      96 |
##           |      0.440 |      0.082 |      |
## -----|-----|-----|-----|
##      True |      15 |      73 |      88 |
##           |      0.082 |      0.397 |      |
## -----|-----|-----|-----|
## Column Total |      96 |      88 |      184 |
## -----|-----|-----|-----|
##
##
```

DEB RNA no FAIMS, charge state 2-7

Now, let us test KNN, C5, and RIPPER models on unknown data: Ecoli DEB no FAIMS runs

Data setup DEB RNA Cno FAIMS charge state 2-7

```
DEB_RNA_no_FAIMS_KNN <- read_delim("AWulf_090919_Ecoli_DEB_S100_noFAIMS_charge_state27_table.csv",
  delim = "\t")
```

```
## Parsed with column specification:
## cols(
##   .default = col_double(),
##   sequence = col_character(),
##   accessions = col_character(),
##   `RNPxl:NT` = col_character(),
##   `RNPxl:RNA` = col_character(),
##   `RNPxl:best_localization` = col_character(),
##   `RNPxl:localization_scores` = col_character(),
##   protein_references = col_character(),
##   target_decoy = col_character(),
##   `PeakAnnotations(mz|intensity|charge|annotation;)` = col_character()
## )
```

See spec(...) for full column specifications.

```
names(DEB_RNA_no_FAIMS_KNN) <- gsub("\\.", "_", names(DEB_RNA_no_FAIMS_KNN))
names(DEB_RNA_no_FAIMS_KNN) <- gsub("\\:", "_", names(DEB_RNA_no_FAIMS_KNN))
names(DEB_RNA_no_FAIMS_KNN) <- gsub("\\ ", "_", names(DEB_RNA_no_FAIMS_KNN))
names(DEB_RNA_no_FAIMS_KNN) <- gsub("\\|", "_", names(DEB_RNA_no_FAIMS_KNN))
DEB_RNA_no_FAIMS_KNN$XLinker <- "DEB"
DEB_RNA_no_FAIMS_KNN$Nucleotides <- "RNA"
DEB_RNA_no_FAIMS_KNN$Sample <- "S100"
DEB_RNA_no_FAIMS_KNN <- DEB_RNA_no_FAIMS_KNN[DEB_RNA_no_FAIMS_KNN$RNPxl_RNA != "none",
] %>% filter(target_decoy == "target")
DEB_RNA_no_FAIMS_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_no_FAIMS_KNN$sequence))
```



```

DEB_RNA_no_FAIMS_KNN$prepPep <- gsub("Oxidation", "", as.character(DEB_RNA_no_FAIMS_KNN$prepPep))
DEB_RNA_no_FAIMS_KNN$prepPep <- gsub("Phospho", "", as.character(DEB_RNA_no_FAIMS_KNN$prepPep))
DEB_RNA_no_FAIMS_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_no_FAIMS_KNN$prepPep)
DEB_RNA_no_FAIMS_KNN$prepPep <- gsub("\\.\\.*", "", DEB_RNA_no_FAIMS_KNN$prepPep)
DEB_RNA_no_FAIMS_KNN$Pepseq <- DEB_RNA_no_FAIMS_KNN$prepPep
DEB_RNA_no_FAIMS_KNN$Peplength <- nchar(DEB_RNA_no_FAIMS_KNN$Pepseq, type = "chars")
DEB_RNA_no_FAIMS_KNN$aa_class_charged <- str_count(DEB_RNA_no_FAIMS_KNN$Pepseq, paste("R|K|D|E",
collapse = "|"))
DEB_RNA_no_FAIMS_KNN$aa_class_polar <- str_count(DEB_RNA_no_FAIMS_KNN$Pepseq, paste("Q|N|H|S|T|Y|C|W",
collapse = "|"))
DEB_RNA_no_FAIMS_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_no_FAIMS_KNN$Pepseq,
paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_no_FAIMS_KNN$pI <- round(pI(DEB_RNA_no_FAIMS_KNN$Pepseq), 2)
DEB_RNA_no_FAIMS_KNN$aIndex <- round(aIndex(DEB_RNA_no_FAIMS_KNN$Pepseq), 2)
DEB_RNA_no_FAIMS_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_no_FAIMS_KNN$RNPxl_best_localization)
DEB_RNA_no_FAIMS_KNN$Hydro <- round(hydrophobicity(DEB_RNA_no_FAIMS_KNN$Pepseq, scale = "KyteDoolittle",
2))
DEB_RNA_no_FAIMS_KNN$mw <- round(mw(DEB_RNA_no_FAIMS_KNN$Pepseq, monoisotopic = F),
2)
DEB_RNA_no_FAIMS_KNN$cv <- c("35_45")

names(DEB_RNA_no_FAIMS_KNN)[names(DEB_RNA_no_FAIMS_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_no_FAIMS_KNN)[names(DEB_RNA_no_FAIMS_KNN) == "precursor_error_(ppm)"] <- "precursor_err"
DEB_RNA_no_FAIMS_KNN <- DEB_RNA_no_FAIMS_KNN %>% filter(RNPxl_isPhospho == "0")

DEB_RNA_no_FAIMS_KNN_model <- DEB_RNA_no_FAIMS_KNN %>% select(colnames(ml_DEB_RNA_train_knn))

```

Normalizing

```

DEB_RNA_no_FAIMS_KNN_model_norm <- DEB_RNA_no_FAIMS_KNN_model %>% select_if(is.numeric)
DEB_RNA_no_FAIMS_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_no_FAIMS_KNN_model_norm,
normalize))
DEB_RNA_no_FAIMS_KNN_model_norm[is.na(DEB_RNA_no_FAIMS_KNN_model_norm)] <- 0

```

Classifying DEB RNA FAIMS data

KNN5

```

DEB_RNA_no_FAIMS_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_no_FAIMS_KNN_model_norm,
c1 = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_no_FAIMS_KNN_model$Prediction <- DEB_RNA_no_FAIMS_knn_pred
table(DEB_RNA_no_FAIMS_KNN_model$Prediction)

```

```

##
## False True
## 36190 5951

```

```

DEB_RNA_no_FAIMS_KNN$Prediction_KNN <- DEB_RNA_no_FAIMS_knn_pred

```

C5.0 with boosting and penalty for false positives

penalize False Positives 4 times more than false negatives.

```

matrix_dimensions<- list(c("True", "False"), c("True", "False"))
names(matrix_dimensions) <- c("predicted", "actual")
error_cost <- matrix(c(0,1,4,0), nrow = 2, dimnames = matrix_dimensions)

ml_DEB_RNA_no_FAIMS_test_C5<-DEB_RNA_no_FAIMS_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_no_FAIMS_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_no_FAIMS_test_C5)

ml_DEB_RNA_no_FAIMS_test_C5$Prediction<-C5_DEB_RNA_no_FAIMS_predict_boost_pen
table(ml_DEB_RNA_no_FAIMS_test_C5$Prediction)

##
## False True
## 26334 15807

DEB_RNA_no_FAIMS_KNN$Prediction_C5<-C5_DEB_RNA_no_FAIMS_predict_boost_pen

```

RIPPER

```

RIPPER_DEB_RNA_no_FAIMS_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_no_FAIMS_test_C5)
ml_DEB_RNA_no_FAIMS_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_no_FAIMS_predict
table(ml_DEB_RNA_no_FAIMS_test_C5$Prediction_RIPPER)

##
## False True
## 23666 18475

DEB_RNA_no_FAIMS_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_no_FAIMS_predict

```

collapsed Proteins

S100 no FAIMS charge state 2-7 KNN

```

d<-DEB_RNA_no_FAIMS_KNN %>%
  filter(Prediction_KNN == "True") %>%
  select(accessions) %>%
  distinct()
nrow(d)

## [1] 2639

write_excel_csv(as.data.frame(d), "S100_DEB_RNA_no_FAIMS_27_accessions.csv")

```

S100 FAIMS FAIMS charge state 2-7 C5.0

```

nrow(DEB_RNA_no_FAIMS_KNN %>%
  filter(Prediction_C5 == "True") %>%
  select(accessions) %>%
  distinct())

## [1] 3651

```

S100 FAIMS FAIMS charge state 2-7 RIPPER

```
nrow(DEB_RNA_no_FAIMS_KNN %>%  
  filter(Prediction_RIPPER == "True") %>%  
  select(accessions) %>%  
  distinct())
```

```
## [1] 3761
```