DEB RNA FAIMS S30

Load required packages

```
## corrplot 0.84 loaded

##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union

## -- Attaching packages --------------------------------------- tidyverse 1.3.0 --

## v ggplot2 3.3.0     v purrr   0.3.4
## v tidyr   1.0.3     v forcats 0.5.0
## v readr   1.3.1

## -- Conflicts ------------------------------------------ tidyverse_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 metrices (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 isolelectric 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 alphatic index, which is defined as the relative volume of a protein occupied by aliphatic side schains (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 particulary 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

Hydrophibicity index

Molecular weight of peptide

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

Machine learning

Split dataset

Seperate 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 %0 F

```r
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 neighboor classification based on numeric variables, min-max normalized.

```
library(class)
library(gmodels)

knn_drop <- c("Curated", "isotope_error", "RNPxl_a_ion_score", "RNPxl_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", "RNPxl_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 10:56:28 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 |
## -------------|-----------|-----------|-----------|
##
##
```

Classifyers

DEB RNA

One Rule (1R)

RIPPER

```r
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
```

```r
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 FAIMS runs S30

Now, let us test KNN, C5, and RIPPER models on unknown data: Ecoli DEB FAIMS runs

Data setup DEB RNA CV 35_45

```
DEB_RNA_FAIMS_35_45_KNN <- read_delim("AWulf_080221_Ecoli_DEB_S30_FAIMS_35_45_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_FAIMS_35_45_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_35_45_KNN))
names(DEB_RNA_FAIMS_35_45_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_35_45_KNN))
names(DEB_RNA_FAIMS_35_45_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_35_45_KNN))
names(DEB_RNA_FAIMS_35_45_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_35_45_KNN))
DEB_RNA_FAIMS_35_45_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_35_45_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_35_45_KNN$Sample <- "S30"
DEB_RNA_FAIMS_35_45_KNN <- DEB_RNA_FAIMS_35_45_KNN[DEB_RNA_FAIMS_35_45_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_35_45_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_35_45_KNN$sequence
DEB_RNA_FAIMS_35_45_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_35_45_KNN$prepPep
DEB_RNA_FAIMS_35_45_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_35_45_KNN$prepPep))
DEB_RNA_FAIMS_35_45_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_35_45_KNN$prepPep)
DEB_RNA_FAIMS_35_45_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_35_45_KNN$prepPep)
DEB_RNA_FAIMS_35_45_KNN$Pepseq <- DEB_RNA_FAIMS_35_45_KNN$prepPep
DEB_RNA_FAIMS_35_45_KNN$Peplength <- nchar(DEB_RNA_FAIMS_35_45_KNN$Pepseq, type = "chars")
DEB_RNA_FAIMS_35_45_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_35_45_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_35_45_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_35_45_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_35_45_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_35_45_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_35_45_KNN$pI <- round(pI(DEB_RNA_FAIMS_35_45_KNN$Pepseq), 2)
DEB_RNA_FAIMS_35_45_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_35_45_KNN$Pepseq), 2)
DEB_RNA_FAIMS_35_45_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_35_45_KNN$RNPxl_best_localization)
DEB_RNA_FAIMS_35_45_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_35_45_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_35_45_KNN$mw <- round(mw(DEB_RNA_FAIMS_35_45_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_35_45_KNN$cv <- c("35_45")

names(DEB_RNA_FAIMS_35_45_KNN)[names(DEB_RNA_FAIMS_35_45_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_35_45_KNN)[names(DEB_RNA_FAIMS_35_45_KNN) == "precursor_error_(_ppm_)"] <- "precurs
DEB_RNA_FAIMS_35_45_KNN <- DEB_RNA_FAIMS_35_45_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_35_45_KNN_model <- DEB_RNA_FAIMS_35_45_KNN %>% select(colnames(ml_DEB_RNA_train_knn))
```

Normalizing

```
DEB_RNA_FAIMS_35_45_KNN_model_norm <- DEB_RNA_FAIMS_35_45_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_35_45_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_35_45_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_35_45_KNN_model_norm[is.na(DEB_RNA_FAIMS_35_45_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_35_45_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_35_45_KNN_model_
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_35_45_KNN_model$Prediction <- DEB_RNA_FAIMS_35_45_knn_pred
table(DEB_RNA_FAIMS_35_45_KNN_model$Prediction)
```

```
##
## False  True
## 29563  3736
```

```
DEB_RNA_FAIMS_35_45_KNN$Prediction_KNN <- DEB_RNA_FAIMS_35_45_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_FAIMS_35_45_test_C5<-DEB_RNA_FAIMS_35_45_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_35_45_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_35_45_tes

ml_DEB_RNA_FAIMS_35_45_test_C5$Prediction<-C5_DEB_RNA_FAIMS_35_45_predict_boost_pen
table(ml_DEB_RNA_FAIMS_35_45_test_C5$Prediction)
```

```
##
## False  True
## 21276 12023
```

```
DEB_RNA_FAIMS_35_45_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_35_45_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_35_45_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_35_45_test_C5)
ml_DEB_RNA_FAIMS_35_45_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_predict
table(ml_DEB_RNA_FAIMS_35_45_test_C5$Prediction_RIPPER)
```

```
##
## False  True
## 19977 13322
```

```
DEB_RNA_FAIMS_35_45_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_predict
```

Data setup DEB RNA CV 40_50

```
DEB_RNA_FAIMS_40_50_KNN <- read_delim("AWulf_080221_Ecoli_DEB_S30_FAIMS_40_50_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_FAIMS_40_50_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_40_50_KNN))
names(DEB_RNA_FAIMS_40_50_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_40_50_KNN))
names(DEB_RNA_FAIMS_40_50_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_40_50_KNN))
names(DEB_RNA_FAIMS_40_50_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_40_50_KNN))
DEB_RNA_FAIMS_40_50_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_40_50_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_40_50_KNN$Sample <- "S30"
DEB_RNA_FAIMS_40_50_KNN <- DEB_RNA_FAIMS_40_50_KNN[DEB_RNA_FAIMS_40_50_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_40_50_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_40_50_KNN$sequence
DEB_RNA_FAIMS_40_50_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_40_50_KNN$prepPep
DEB_RNA_FAIMS_40_50_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_40_50_KNN$prepPep))
DEB_RNA_FAIMS_40_50_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_40_50_KNN$prepPep)
DEB_RNA_FAIMS_40_50_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_40_50_KNN$prepPep)
DEB_RNA_FAIMS_40_50_KNN$Pepseq <- DEB_RNA_FAIMS_40_50_KNN$prepPep
DEB_RNA_FAIMS_40_50_KNN$Peplength <- nchar(DEB_RNA_FAIMS_40_50_KNN$Pepseq, type = "chars")
DEB_RNA_FAIMS_40_50_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_40_50_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_40_50_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_40_50_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_40_50_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_40_50_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_40_50_KNN$pI <- round(pI(DEB_RNA_FAIMS_40_50_KNN$Pepseq), 2)
DEB_RNA_FAIMS_40_50_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_40_50_KNN$Pepseq), 2)
DEB_RNA_FAIMS_40_50_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_40_50_KNN$RNPxl_best_localization)
DEB_RNA_FAIMS_40_50_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_40_50_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_40_50_KNN$mw <- round(mw(DEB_RNA_FAIMS_40_50_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_40_50_KNN$cv <- c("40_50")

names(DEB_RNA_FAIMS_40_50_KNN)[names(DEB_RNA_FAIMS_40_50_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_40_50_KNN)[names(DEB_RNA_FAIMS_40_50_KNN) == "precursor_error_(_ppm_)"] <- "precurs
DEB_RNA_FAIMS_40_50_KNN <- DEB_RNA_FAIMS_40_50_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_40_50_KNN_model <- DEB_RNA_FAIMS_40_50_KNN %>% select(colnames(ml_DEB_RNA_train_knn))
```

Normalizing

```
DEB_RNA_FAIMS_40_50_KNN_model_norm <- DEB_RNA_FAIMS_40_50_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_40_50_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_40_50_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_40_50_KNN_model_norm[is.na(DEB_RNA_FAIMS_40_50_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_40_50_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_40_50_KNN_model_r
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_40_50_KNN_model$Prediction <- DEB_RNA_FAIMS_40_50_knn_pred
table(DEB_RNA_FAIMS_40_50_KNN_model$Prediction)
```

```
##
## False  True
## 19147  4525
```

```
DEB_RNA_FAIMS_40_50_KNN$Prediction_KNN <- DEB_RNA_FAIMS_40_50_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_FAIMS_40_50_test_C5<-DEB_RNA_FAIMS_40_50_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_40_50_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_40_50_tes

ml_DEB_RNA_FAIMS_40_50_test_C5$Prediction<-C5_DEB_RNA_FAIMS_40_50_predict_boost_pen
table(ml_DEB_RNA_FAIMS_40_50_test_C5$Prediction)
```

```
##
## False  True
## 16113  7559
```

```
DEB_RNA_FAIMS_40_50_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_40_50_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_40_50_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_40_50_test_C5)
ml_DEB_RNA_FAIMS_40_50_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_40_50_predict
table(ml_DEB_RNA_FAIMS_40_50_test_C5$Prediction_RIPPER)
```

```
##
## False  True
## 13420 10252
```

```
DEB_RNA_FAIMS_40_50_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_40_50_predict
```

Data setup DEB RNA CV 55_65

```
DEB_RNA_FAIMS_55_65_KNN <- read_delim("AWulf_080221_Ecoli_DEB_S30_FAIMS_55_65_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.
```

```r
names(DEB_RNA_FAIMS_55_65_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_55_65_KNN))
names(DEB_RNA_FAIMS_55_65_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_55_65_KNN))
names(DEB_RNA_FAIMS_55_65_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_55_65_KNN))
names(DEB_RNA_FAIMS_55_65_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_55_65_KNN))
DEB_RNA_FAIMS_55_65_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_55_65_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_55_65_KNN$Sample <- "S30"
DEB_RNA_FAIMS_55_65_KNN <- DEB_RNA_FAIMS_55_65_KNN[DEB_RNA_FAIMS_55_65_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_55_65_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_55_65_KNN$sequence
DEB_RNA_FAIMS_55_65_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_55_65_KNN$prepPep
DEB_RNA_FAIMS_55_65_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_55_65_KNN$prepPep))
DEB_RNA_FAIMS_55_65_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_55_65_KNN$prepPep)
DEB_RNA_FAIMS_55_65_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_55_65_KNN$prepPep)
DEB_RNA_FAIMS_55_65_KNN$Pepseq <- DEB_RNA_FAIMS_55_65_KNN$prepPep
DEB_RNA_FAIMS_55_65_KNN$Peplength <- nchar(DEB_RNA_FAIMS_55_65_KNN$Pepseq, type = "chars")
DEB_RNA_FAIMS_55_65_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_55_65_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_55_65_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_55_65_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_55_65_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_55_65_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_55_65_KNN$pI <- round(pI(DEB_RNA_FAIMS_55_65_KNN$Pepseq), 2)
DEB_RNA_FAIMS_55_65_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_55_65_KNN$Pepseq), 2)
DEB_RNA_FAIMS_55_65_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_55_65_KNN$RNPxl_best_localization)
DEB_RNA_FAIMS_55_65_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_55_65_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_55_65_KNN$mw <- round(mw(DEB_RNA_FAIMS_55_65_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_55_65_KNN$cv <- c("55_65")

names(DEB_RNA_FAIMS_55_65_KNN)[names(DEB_RNA_FAIMS_55_65_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_55_65_KNN)[names(DEB_RNA_FAIMS_55_65_KNN) == "precursor_error_(_ppm_)"] <- "precurs
DEB_RNA_FAIMS_55_65_KNN <- DEB_RNA_FAIMS_55_65_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_55_65_KNN_model <- DEB_RNA_FAIMS_55_65_KNN %>% select(colnames(ml_DEB_RNA_train_knn))
```

Normalizing

```
DEB_RNA_FAIMS_55_65_KNN_model_norm <- DEB_RNA_FAIMS_55_65_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_55_65_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_55_65_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_55_65_KNN_model_norm[is.na(DEB_RNA_FAIMS_55_65_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_55_65_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_55_65_KNN_model_n
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_55_65_KNN_model$Prediction <- DEB_RNA_FAIMS_55_65_knn_pred
table(DEB_RNA_FAIMS_55_65_KNN_model$Prediction)
```

```
##
## False  True
## 33883  4060
```

```
DEB_RNA_FAIMS_55_65_KNN$Prediction_KNN <- DEB_RNA_FAIMS_55_65_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_FAIMS_55_65_test_C5<-DEB_RNA_FAIMS_55_65_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_55_65_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_55_65_tes

ml_DEB_RNA_FAIMS_55_65_test_C5$Prediction<-C5_DEB_RNA_FAIMS_55_65_predict_boost_pen
table(ml_DEB_RNA_FAIMS_55_65_test_C5$Prediction)
```

```
##
## False  True
## 22945 14998
```

```
DEB_RNA_FAIMS_55_65_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_55_65_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_55_65_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_55_65_test_C5)
ml_DEB_RNA_FAIMS_55_65_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_55_65_predict
table(ml_DEB_RNA_FAIMS_55_65_test_C5$Prediction_RIPPER)
```

```
##
## False  True
## 19702 18241
```

```
DEB_RNA_FAIMS_55_65_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_55_65_predict
```

Data setup DEB RNA CV 60_70

```
DEB_RNA_FAIMS_60_70_KNN <- read_delim("AWulf_080221_Ecoli_DEB_S30_FAIMS_60_70_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_FAIMS_60_70_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_60_70_KNN))
names(DEB_RNA_FAIMS_60_70_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_60_70_KNN))
names(DEB_RNA_FAIMS_60_70_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_60_70_KNN))
names(DEB_RNA_FAIMS_60_70_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_60_70_KNN))
DEB_RNA_FAIMS_60_70_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_60_70_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_60_70_KNN$Sample <- "S30"
DEB_RNA_FAIMS_60_70_KNN <- DEB_RNA_FAIMS_60_70_KNN[DEB_RNA_FAIMS_60_70_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_60_70_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_60_70_KNN$sequence
DEB_RNA_FAIMS_60_70_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_60_70_KNN$prepPep
DEB_RNA_FAIMS_60_70_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_60_70_KNN$prepPep))
DEB_RNA_FAIMS_60_70_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_60_70_KNN$prepPep)
DEB_RNA_FAIMS_60_70_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_60_70_KNN$prepPep)
DEB_RNA_FAIMS_60_70_KNN$Pepseq <- DEB_RNA_FAIMS_60_70_KNN$prepPep
DEB_RNA_FAIMS_60_70_KNN$Peplength <- nchar(DEB_RNA_FAIMS_60_70_KNN$Pepseq, type = "chars")
DEB_RNA_FAIMS_60_70_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_60_70_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_60_70_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_60_70_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_60_70_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_60_70_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_60_70_KNN$pI <- round(pI(DEB_RNA_FAIMS_60_70_KNN$Pepseq), 2)
DEB_RNA_FAIMS_60_70_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_60_70_KNN$Pepseq), 2)
DEB_RNA_FAIMS_60_70_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_60_70_KNN$RNPxl_best_localization)
DEB_RNA_FAIMS_60_70_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_60_70_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_60_70_KNN$mw <- round(mw(DEB_RNA_FAIMS_60_70_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_60_70_KNN$cv <- c("60_70")

names(DEB_RNA_FAIMS_60_70_KNN)[names(DEB_RNA_FAIMS_60_70_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_60_70_KNN)[names(DEB_RNA_FAIMS_60_70_KNN) == "precursor_error_(_ppm_)"] <- "precurs
DEB_RNA_FAIMS_60_70_KNN <- DEB_RNA_FAIMS_60_70_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_60_70_KNN_model <- DEB_RNA_FAIMS_60_70_KNN %>% select(colnames(ml_DEB_RNA_train_knn))
```

Normalizing

```
DEB_RNA_FAIMS_60_70_KNN_model_norm <- DEB_RNA_FAIMS_60_70_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_60_70_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_60_70_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_60_70_KNN_model_norm[is.na(DEB_RNA_FAIMS_60_70_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_60_70_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_60_70_KNN_model_
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_60_70_KNN_model$Prediction <- DEB_RNA_FAIMS_60_70_knn_pred
table(DEB_RNA_FAIMS_60_70_KNN_model$Prediction)
```

```
##
## False  True
## 29819  3837
```

```
DEB_RNA_FAIMS_60_70_KNN$Prediction_KNN <- DEB_RNA_FAIMS_60_70_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_FAIMS_60_70_test_C5<-DEB_RNA_FAIMS_60_70_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_60_70_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_60_70_te

ml_DEB_RNA_FAIMS_60_70_test_C5$Prediction<-C5_DEB_RNA_FAIMS_60_70_predict_boost_pen
table(ml_DEB_RNA_FAIMS_60_70_test_C5$Prediction)
```

```
##
## False  True
## 18825 14831
```

```
DEB_RNA_FAIMS_60_70_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_60_70_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_60_70_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_60_70_test_C5)
ml_DEB_RNA_FAIMS_60_70_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_60_70_predict
table(ml_DEB_RNA_FAIMS_60_70_test_C5$Prediction_RIPPER)
```

```
##
```

```
## False  True
## 15820 17836
```

```
DEB_RNA_FAIMS_60_70_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_60_70_predict
```

DEB RNA FAIMS runs S30

Now, let us test KNN, C5, and RIPPER models on unknown data: Ecoli DEB FAIMS runs

Data setup DEB RNA CV 35_45

```
DEB_RNA_FAIMS_35_45_KNN <- read_delim("AWulf_080221_Ecoli_DEB_S30_FAIMS_35_45_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_FAIMS_35_45_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_35_45_KNN))
names(DEB_RNA_FAIMS_35_45_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_35_45_KNN))
names(DEB_RNA_FAIMS_35_45_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_35_45_KNN))
names(DEB_RNA_FAIMS_35_45_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_35_45_KNN))
DEB_RNA_FAIMS_35_45_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_35_45_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_35_45_KNN$Sample <- "30"
DEB_RNA_FAIMS_35_45_KNN <- DEB_RNA_FAIMS_35_45_KNN[DEB_RNA_FAIMS_35_45_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_35_45_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_35_45_KNN$sequence
DEB_RNA_FAIMS_35_45_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_35_45_KNN$prepPep
DEB_RNA_FAIMS_35_45_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_35_45_KNN$prepPep))
DEB_RNA_FAIMS_35_45_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_35_45_KNN$prepPep)
DEB_RNA_FAIMS_35_45_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_35_45_KNN$prepPep)
DEB_RNA_FAIMS_35_45_KNN$Pepseq <- DEB_RNA_FAIMS_35_45_KNN$prepPep
DEB_RNA_FAIMS_35_45_KNN$Peplength <- nchar(DEB_RNA_FAIMS_35_45_KNN$Pepseq, type = "chars")
DEB_RNA_FAIMS_35_45_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_35_45_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_35_45_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_35_45_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_35_45_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_35_45_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_35_45_KNN$pI <- round(pI(DEB_RNA_FAIMS_35_45_KNN$Pepseq), 2)
DEB_RNA_FAIMS_35_45_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_35_45_KNN$Pepseq), 2)
DEB_RNA_FAIMS_35_45_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_35_45_KNN$RNPxl_best_localization)
```

```
DEB_RNA_FAIMS_35_45_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_35_45_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_35_45_KNN$mw <- round(mw(DEB_RNA_FAIMS_35_45_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_35_45_KNN$cv <- c("35_45")

names(DEB_RNA_FAIMS_35_45_KNN)[names(DEB_RNA_FAIMS_35_45_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_35_45_KNN)[names(DEB_RNA_FAIMS_35_45_KNN) == "precursor_error_(_ppm_)"] <- "precurs
DEB_RNA_FAIMS_35_45_KNN <- DEB_RNA_FAIMS_35_45_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_35_45_KNN_model <- DEB_RNA_FAIMS_35_45_KNN %>% select(colnames(ml_DEB_RNA_train_knn))
```

Normalizing

```
DEB_RNA_FAIMS_35_45_KNN_model_norm <- DEB_RNA_FAIMS_35_45_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_35_45_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_35_45_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_35_45_KNN_model_norm[is.na(DEB_RNA_FAIMS_35_45_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_35_45_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_35_45_KNN_model_
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_35_45_KNN_model$Prediction <- DEB_RNA_FAIMS_35_45_knn_pred
table(DEB_RNA_FAIMS_35_45_KNN_model$Prediction)
```

```
##
## False  True
## 29570  3729
```

```
DEB_RNA_FAIMS_35_45_KNN$Prediction_KNN <- DEB_RNA_FAIMS_35_45_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_FAIMS_35_45_test_C5<-DEB_RNA_FAIMS_35_45_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_35_45_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_35_45_te

ml_DEB_RNA_FAIMS_35_45_test_C5$Prediction<-C5_DEB_RNA_FAIMS_35_45_predict_boost_pen
table(ml_DEB_RNA_FAIMS_35_45_test_C5$Prediction)
```

```
##
## False  True
## 21276 12023
```

```
DEB_RNA_FAIMS_35_45_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_35_45_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_35_45_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_35_45_test_C5)
ml_DEB_RNA_FAIMS_35_45_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_predict
table(ml_DEB_RNA_FAIMS_35_45_test_C5$Prediction_RIPPER)
```

```
##
## False  True
## 19977 13322
```

```
DEB_RNA_FAIMS_35_45_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_predict
```

Data setup DEB RNA CV 40_50

```
DEB_RNA_FAIMS_40_50_KNN <- read_delim("AWulf_080221_Ecoli_DEB_S30_FAIMS_40_50_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_FAIMS_40_50_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_40_50_KNN))
names(DEB_RNA_FAIMS_40_50_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_40_50_KNN))
names(DEB_RNA_FAIMS_40_50_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_40_50_KNN))
names(DEB_RNA_FAIMS_40_50_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_40_50_KNN))
DEB_RNA_FAIMS_40_50_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_40_50_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_40_50_KNN$Sample <- "S30"
DEB_RNA_FAIMS_40_50_KNN <- DEB_RNA_FAIMS_40_50_KNN[DEB_RNA_FAIMS_40_50_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_40_50_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_40_50_KNN$sequence
DEB_RNA_FAIMS_40_50_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_40_50_KNN$prepPep
DEB_RNA_FAIMS_40_50_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_40_50_KNN$prepPep))
DEB_RNA_FAIMS_40_50_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_40_50_KNN$prepPep)
DEB_RNA_FAIMS_40_50_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_40_50_KNN$prepPep)
DEB_RNA_FAIMS_40_50_KNN$Pepseq <- DEB_RNA_FAIMS_40_50_KNN$prepPep
DEB_RNA_FAIMS_40_50_KNN$Peplength <- nchar(DEB_RNA_FAIMS_40_50_KNN$Pepseq, type = "chars")
DEB_RNA_FAIMS_40_50_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_40_50_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_40_50_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_40_50_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_40_50_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_40_50_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_40_50_KNN$pI <- round(pI(DEB_RNA_FAIMS_40_50_KNN$Pepseq), 2)
DEB_RNA_FAIMS_40_50_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_40_50_KNN$Pepseq), 2)
DEB_RNA_FAIMS_40_50_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_40_50_KNN$RNPxl_best_localization)
DEB_RNA_FAIMS_40_50_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_40_50_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_40_50_KNN$mw <- round(mw(DEB_RNA_FAIMS_40_50_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_40_50_KNN$cv <- c("40_50")

names(DEB_RNA_FAIMS_40_50_KNN)[names(DEB_RNA_FAIMS_40_50_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_40_50_KNN)[names(DEB_RNA_FAIMS_40_50_KNN) == "precursor_error_(_ppm_)"] <- "precurso
DEB_RNA_FAIMS_40_50_KNN <- DEB_RNA_FAIMS_40_50_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_40_50_KNN_model <- DEB_RNA_FAIMS_40_50_KNN %>% select(colnames(ml_DEB_RNA_train_knn))
```

Normalizing

```
DEB_RNA_FAIMS_40_50_KNN_model_norm <- DEB_RNA_FAIMS_40_50_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_40_50_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_40_50_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_40_50_KNN_model_norm[is.na(DEB_RNA_FAIMS_40_50_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_40_50_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_40_50_KNN_model_r
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_40_50_KNN_model$Prediction <- DEB_RNA_FAIMS_40_50_knn_pred
table(DEB_RNA_FAIMS_40_50_KNN_model$Prediction)
```

```
##
## False  True
## 19144  4528
```

```
DEB_RNA_FAIMS_40_50_KNN$Prediction_KNN <- DEB_RNA_FAIMS_40_50_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_FAIMS_40_50_test_C5<-DEB_RNA_FAIMS_40_50_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_40_50_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_40_50_tes
ml_DEB_RNA_FAIMS_40_50_test_C5$Prediction<-C5_DEB_RNA_FAIMS_40_50_predict_boost_pen
table(ml_DEB_RNA_FAIMS_40_50_test_C5$Prediction)
```

```
##
## False  True
## 16113  7559
```

```
DEB_RNA_FAIMS_40_50_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_40_50_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_40_50_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_40_50_test_C5)
ml_DEB_RNA_FAIMS_40_50_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_40_50_predict
table(ml_DEB_RNA_FAIMS_40_50_test_C5$Prediction_RIPPER)
```

```
##
## False  True
## 13420 10252
```

```
DEB_RNA_FAIMS_40_50_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_40_50_predict
```

Data setup DEB RNA CV 55_65

```
DEB_RNA_FAIMS_55_65_KNN <- read_delim("AWulf_080221_Ecoli_DEB_S30_FAIMS_55_65_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_FAIMS_55_65_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_55_65_KNN))
names(DEB_RNA_FAIMS_55_65_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_55_65_KNN))
names(DEB_RNA_FAIMS_55_65_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_55_65_KNN))
names(DEB_RNA_FAIMS_55_65_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_55_65_KNN))
DEB_RNA_FAIMS_55_65_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_55_65_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_55_65_KNN$Sample <- "S30"
DEB_RNA_FAIMS_55_65_KNN <- DEB_RNA_FAIMS_55_65_KNN[DEB_RNA_FAIMS_55_65_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_55_65_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_55_65_KNN$sequence
DEB_RNA_FAIMS_55_65_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_55_65_KNN$prepPep
DEB_RNA_FAIMS_55_65_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_55_65_KNN$prepPep))
DEB_RNA_FAIMS_55_65_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_55_65_KNN$prepPep)
```

```
DEB_RNA_FAIMS_55_65_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_55_65_KNN$prepPep)
DEB_RNA_FAIMS_55_65_KNN$Pepseq <- DEB_RNA_FAIMS_55_65_KNN$prepPep
DEB_RNA_FAIMS_55_65_KNN$Peplength <- nchar(DEB_RNA_FAIMS_55_65_KNN$Pepseq, type = "chars")
DEB_RNA_FAIMS_55_65_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_55_65_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_55_65_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_55_65_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_55_65_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_55_65_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_55_65_KNN$pI <- round(pI(DEB_RNA_FAIMS_55_65_KNN$Pepseq), 2)
DEB_RNA_FAIMS_55_65_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_55_65_KNN$Pepseq), 2)
DEB_RNA_FAIMS_55_65_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_55_65_KNN$RNPxl_best_localization)
DEB_RNA_FAIMS_55_65_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_55_65_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_55_65_KNN$mw <- round(mw(DEB_RNA_FAIMS_55_65_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_55_65_KNN$cv <- c("55_65")

names(DEB_RNA_FAIMS_55_65_KNN)[names(DEB_RNA_FAIMS_55_65_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_55_65_KNN)[names(DEB_RNA_FAIMS_55_65_KNN) == "precursor_error_(_ppm_)"] <- "precurs
DEB_RNA_FAIMS_55_65_KNN <- DEB_RNA_FAIMS_55_65_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_55_65_KNN_model <- DEB_RNA_FAIMS_55_65_KNN %>% select(colnames(ml_DEB_RNA_train_knn))
```

Normalizing

```
DEB_RNA_FAIMS_55_65_KNN_model_norm <- DEB_RNA_FAIMS_55_65_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_55_65_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_55_65_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_55_65_KNN_model_norm[is.na(DEB_RNA_FAIMS_55_65_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_55_65_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_55_65_KNN_model_
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_55_65_KNN_model$Prediction <- DEB_RNA_FAIMS_55_65_knn_pred
table(DEB_RNA_FAIMS_55_65_KNN_model$Prediction)
```

```
##
## False  True
## 33879  4064
```

```
DEB_RNA_FAIMS_55_65_KNN$Prediction_KNN <- DEB_RNA_FAIMS_55_65_knn_pred

d <- DEB_RNA_FAIMS_55_65_KNN %>% filter(Prediction_KNN == "True") %>% select(accessions) %>%
    distinct()
nrow(d)
```

```
## [1] 2093
```

```
write_excel_csv(as.data.frame(d), "S30_DEB_RNA_FAIMS_55_65_accessions.csv")
```

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_FAIMS_55_65_test_C5<-DEB_RNA_FAIMS_55_65_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_55_65_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_55_65_te

ml_DEB_RNA_FAIMS_55_65_test_C5$Prediction<-C5_DEB_RNA_FAIMS_55_65_predict_boost_pen
table(ml_DEB_RNA_FAIMS_55_65_test_C5$Prediction)
```

```
##
## False  True
## 22945 14998
```

```
DEB_RNA_FAIMS_55_65_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_55_65_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_55_65_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_55_65_test_C5)
ml_DEB_RNA_FAIMS_55_65_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_55_65_predict
table(ml_DEB_RNA_FAIMS_55_65_test_C5$Prediction_RIPPER)
```

```
##
## False  True
## 19702 18241
```

```
DEB_RNA_FAIMS_55_65_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_55_65_predict
```

Data setup DEB RNA CV 60_70

```
DEB_RNA_FAIMS_60_70_KNN <- read_delim("AWulf_080221_Ecoli_DEB_S30_FAIMS_60_70_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.
```

```r
names(DEB_RNA_FAIMS_60_70_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_60_70_KNN))
names(DEB_RNA_FAIMS_60_70_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_60_70_KNN))
names(DEB_RNA_FAIMS_60_70_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_60_70_KNN))
names(DEB_RNA_FAIMS_60_70_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_60_70_KNN))
DEB_RNA_FAIMS_60_70_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_60_70_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_60_70_KNN$Sample <- "S30"
DEB_RNA_FAIMS_60_70_KNN <- DEB_RNA_FAIMS_60_70_KNN[DEB_RNA_FAIMS_60_70_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_60_70_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_60_70_KNN$sequence
DEB_RNA_FAIMS_60_70_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_60_70_KNN$prepPep
DEB_RNA_FAIMS_60_70_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_60_70_KNN$prepPep))
DEB_RNA_FAIMS_60_70_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_60_70_KNN$prepPep)
DEB_RNA_FAIMS_60_70_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_60_70_KNN$prepPep)
DEB_RNA_FAIMS_60_70_KNN$Pepseq <- DEB_RNA_FAIMS_60_70_KNN$prepPep
DEB_RNA_FAIMS_60_70_KNN$Peplength <- nchar(DEB_RNA_FAIMS_60_70_KNN$Pepseq, type = "chars")
DEB_RNA_FAIMS_60_70_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_60_70_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_60_70_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_60_70_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_60_70_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_60_70_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_60_70_KNN$pI <- round(pI(DEB_RNA_FAIMS_60_70_KNN$Pepseq), 2)
DEB_RNA_FAIMS_60_70_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_60_70_KNN$Pepseq), 2)
DEB_RNA_FAIMS_60_70_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_60_70_KNN$RNPxl_best_localization)
DEB_RNA_FAIMS_60_70_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_60_70_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_60_70_KNN$mw <- round(mw(DEB_RNA_FAIMS_60_70_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_60_70_KNN$cv <- c("60_70")

names(DEB_RNA_FAIMS_60_70_KNN)[names(DEB_RNA_FAIMS_60_70_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_60_70_KNN)[names(DEB_RNA_FAIMS_60_70_KNN) == "precursor_error_(_ppm_)"] <- "precurs
DEB_RNA_FAIMS_60_70_KNN <- DEB_RNA_FAIMS_60_70_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_60_70_KNN_model <- DEB_RNA_FAIMS_60_70_KNN %>% select(colnames(ml_DEB_RNA_train_knn))
```

Normalizing

```r
DEB_RNA_FAIMS_60_70_KNN_model_norm <- DEB_RNA_FAIMS_60_70_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_60_70_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_60_70_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_60_70_KNN_model_norm[is.na(DEB_RNA_FAIMS_60_70_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```r
DEB_RNA_FAIMS_60_70_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_60_70_KNN_model_
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_60_70_KNN_model$Prediction <- DEB_RNA_FAIMS_60_70_knn_pred
```

```
DEB_RNA_FAIMS_60_70_KNN$Prediction_KNN <- DEB_RNA_FAIMS_60_70_knn_pred
table(DEB_RNA_FAIMS_60_70_KNN_model$Prediction)
```

```
##
## False  True
## 29827  3829
```

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_FAIMS_60_70_test_C5<-DEB_RNA_FAIMS_60_70_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_60_70_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_60_70_tes
ml_DEB_RNA_FAIMS_60_70_test_C5$Prediction<-C5_DEB_RNA_FAIMS_60_70_predict_boost_pen
DEB_RNA_FAIMS_60_70_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_60_70_predict_boost_pen
table(ml_DEB_RNA_FAIMS_60_70_test_C5$Prediction)
```

```
##
## False  True
## 18825 14831
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_60_70_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_60_70_test_C5)
ml_DEB_RNA_FAIMS_60_70_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_60_70_predict
table(ml_DEB_RNA_FAIMS_60_70_test_C5$Prediction_RIPPER)
```

```
##
## False  True
## 15820 17836
```

```
DEB_RNA_FAIMS_60_70_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_60_70_predict
```

collapsed Proteins

S30 FAIMS 35_45 KNN

```
nrow(DEB_RNA_FAIMS_35_45_KNN %>%
  filter(Prediction_KNN == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 2012
```

S30 FAIMS 35_45 C5.0

```
nrow(DEB_RNA_FAIMS_35_45_KNN %>%
  filter(Prediction_C5 == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 3364
```

S30 FAIMS 35_45 RIPPER

```
nrow(DEB_RNA_FAIMS_35_45_KNN %>%
  filter(Prediction_RIPPER == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 3451
```

S30 FAIMS 40_50 KNN

```
nrow(DEB_RNA_FAIMS_40_50_KNN %>%
  filter(Prediction_KNN == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 1947
```

S30 FAIMS 40_50 C5.0

```
nrow(DEB_RNA_FAIMS_40_50_KNN %>%
  filter(Prediction_C5 == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 2612
```

S30 FAIMS 40_50 RIPPER

```
nrow(DEB_RNA_FAIMS_40_50_KNN %>%
  filter(Prediction_RIPPER == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 2967
```

S30 FAIMS 55_65 KNN

```
nrow(DEB_RNA_FAIMS_55_65_KNN %>%
  filter(Prediction_KNN == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 2093
```

S30 FAIMS 55_65 C5.0

```
nrow(DEB_RNA_FAIMS_55_65_KNN %>%
  filter(Prediction_C5 == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 3540
```

S30 FAIMS 55_65 RIPPER

```
nrow(DEB_RNA_FAIMS_55_65_KNN %>%
  filter(Prediction_RIPPER == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 3736
```

S30 FAIMS 60_70 KNN

```
nrow(DEB_RNA_FAIMS_60_70_KNN %>%
  filter(Prediction_KNN == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 2066
```

S30 FAIMS 60_70 C5.0

```
nrow(DEB_RNA_FAIMS_60_70_KNN %>%
  filter(Prediction_C5 == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 3506
```

S30 FAIMS 60_70 RIPPER

```
nrow(DEB_RNA_FAIMS_60_70_KNN %>%
  filter(Prediction_RIPPER == "True") %>%
  select(accessions) %>%
  distinct())
```

```
## [1] 3703
```

Data setup DEB RNA CV 35_45_55

```
DEB_RNA_FAIMS_35_45_55_KNN <- read_delim("AWulf_050919_Ecoli_DEB_S30_FAIMS_35_45_55_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_FAIMS_35_45_55_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_35_45_55_KNN))
names(DEB_RNA_FAIMS_35_45_55_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_35_45_55_KNN))
names(DEB_RNA_FAIMS_35_45_55_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_35_45_55_KNN))
names(DEB_RNA_FAIMS_35_45_55_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_35_45_55_KNN))
DEB_RNA_FAIMS_35_45_55_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_35_45_55_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_35_45_55_KNN$Sample <- "S30"
DEB_RNA_FAIMS_35_45_55_KNN <- DEB_RNA_FAIMS_35_45_55_KNN[DEB_RNA_FAIMS_35_45_55_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_35_45_55_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_35_45_55_KNN$se
DEB_RNA_FAIMS_35_45_55_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_35_45_55_KNN$p
DEB_RNA_FAIMS_35_45_55_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_35_45_55_KNN$prep
DEB_RNA_FAIMS_35_45_55_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_35_45_55_KNN$prepPep)
DEB_RNA_FAIMS_35_45_55_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_35_45_55_KNN$prepPep)
DEB_RNA_FAIMS_35_45_55_KNN$Pepseq <- DEB_RNA_FAIMS_35_45_55_KNN$prepPep
DEB_RNA_FAIMS_35_45_55_KNN$Peplength <- nchar(DEB_RNA_FAIMS_35_45_55_KNN$Pepseq,
    type = "chars")
DEB_RNA_FAIMS_35_45_55_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_35_45_55_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_35_45_55_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_35_45_55_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_35_45_55_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_35_45_55_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_35_45_55_KNN$pI <- round(pI(DEB_RNA_FAIMS_35_45_55_KNN$Pepseq), 2)
DEB_RNA_FAIMS_35_45_55_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_35_45_55_KNN$Pepseq),
    2)
DEB_RNA_FAIMS_35_45_55_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_35_45_55_KNN$RNPxl_best_localizati
DEB_RNA_FAIMS_35_45_55_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_35_45_55_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_35_45_55_KNN$mw <- round(mw(DEB_RNA_FAIMS_35_45_55_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_35_45_55_KNN$cv <- c("35_45")

names(DEB_RNA_FAIMS_35_45_55_KNN)[names(DEB_RNA_FAIMS_35_45_55_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_35_45_55_KNN)[names(DEB_RNA_FAIMS_35_45_55_KNN) == "precursor_error_(_ppm_)"] <- "p
DEB_RNA_FAIMS_35_45_55_KNN <- DEB_RNA_FAIMS_35_45_55_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_35_45_55_KNN_model <- DEB_RNA_FAIMS_35_45_55_KNN %>% select(colnames(ml_DEB_RNA_train_knn
```

Normalizing

```
DEB_RNA_FAIMS_35_45_55_KNN_model_norm <- DEB_RNA_FAIMS_35_45_55_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_35_45_55_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_35_45_55_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_35_45_55_KNN_model_norm[is.na(DEB_RNA_FAIMS_35_45_55_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_35_45_55_knn_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_35_45_55_KNN_m
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_35_45_55_KNN_model$Prediction <- DEB_RNA_FAIMS_35_45_55_knn_pred
table(DEB_RNA_FAIMS_35_45_55_KNN_model$Prediction)
```

```
##
## False  True
## 31917  5022
```

```
DEB_RNA_FAIMS_35_45_55_KNN$Prediction_KNN <- DEB_RNA_FAIMS_35_45_55_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_FAIMS_35_45_55_test_C5<-DEB_RNA_FAIMS_35_45_55_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_35_45

ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction<-C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen
table(ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction)
```

```
##
## False  True
## 23372 13567
```

```
DEB_RNA_FAIMS_35_45_55_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_35_45_55_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_35_45_55_test_C5
ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_55_predict
table(ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction_RIPPER)
```

```
##
## False  True
## 20432 16507
```

```
DEB_RNA_FAIMS_35_45_55_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_55_predict
```

Data setup DEB RNA CV 40_50_60

```
DEB_RNA_FAIMS_40_50_60_KNN <- read_delim("AWulf_060919_Ecoli_DEB_S30_FAIMS_40_50_60_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_FAIMS_40_50_60_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_40_50_60_KNN))
names(DEB_RNA_FAIMS_40_50_60_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_40_50_60_KNN))
names(DEB_RNA_FAIMS_40_50_60_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_40_50_60_KNN))
names(DEB_RNA_FAIMS_40_50_60_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_40_50_60_KNN))
DEB_RNA_FAIMS_40_50_60_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_40_50_60_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_40_50_60_KNN$Sample <- "S30"
DEB_RNA_FAIMS_40_50_60_KNN <- DEB_RNA_FAIMS_40_50_60_KNN[DEB_RNA_FAIMS_40_50_60_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_40_50_60_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_40_50_60_KNN$se
DEB_RNA_FAIMS_40_50_60_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_40_50_60_KNN$p
DEB_RNA_FAIMS_40_50_60_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_40_50_60_KNN$pre
DEB_RNA_FAIMS_40_50_60_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_40_50_60_KNN$prepPep)
DEB_RNA_FAIMS_40_50_60_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_40_50_60_KNN$prepPep)
DEB_RNA_FAIMS_40_50_60_KNN$Pepseq <- DEB_RNA_FAIMS_40_50_60_KNN$prepPep
DEB_RNA_FAIMS_40_50_60_KNN$Peplength <- nchar(DEB_RNA_FAIMS_40_50_60_KNN$Pepseq,
    type = "chars")
DEB_RNA_FAIMS_40_50_60_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_40_50_60_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_40_50_60_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_40_50_60_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_40_50_60_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_40_50_60_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_40_50_60_KNN$pI <- round(pI(DEB_RNA_FAIMS_40_50_60_KNN$Pepseq), 2)
DEB_RNA_FAIMS_40_50_60_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_40_50_60_KNN$Pepseq),
    2)
DEB_RNA_FAIMS_40_50_60_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_40_50_60_KNN$RNPxl_best_localizat
DEB_RNA_FAIMS_40_50_60_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_40_50_60_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_40_50_60_KNN$mw <- round(mw(DEB_RNA_FAIMS_40_50_60_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_40_50_60_KNN$cv <- c("35_45")

names(DEB_RNA_FAIMS_40_50_60_KNN)[names(DEB_RNA_FAIMS_40_50_60_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_40_50_60_KNN)[names(DEB_RNA_FAIMS_40_50_60_KNN) == "precursor_error_(_ppm_)"] <- "p
DEB_RNA_FAIMS_40_50_60_KNN <- DEB_RNA_FAIMS_40_50_60_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_40_50_60_KNN_model <- DEB_RNA_FAIMS_40_50_60_KNN %>% select(colnames(ml_DEB_RNA_train_knn
```

Normalizing

```
DEB_RNA_FAIMS_40_50_60_KNN_model_norm <- DEB_RNA_FAIMS_40_50_60_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_40_50_60_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_40_50_60_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_40_50_60_KNN_model_norm[is.na(DEB_RNA_FAIMS_40_50_60_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_40_50_60_KNN_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_40_50_60_KNN_
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_40_50_60_KNN_model$Prediction <- DEB_RNA_FAIMS_40_50_60_KNN_pred
table(DEB_RNA_FAIMS_40_50_60_KNN_model$Prediction)
```

```
##
## False  True
## 30946  5225
```

```
DEB_RNA_FAIMS_40_50_60_KNN$Prediction_KNN <- DEB_RNA_FAIMS_40_50_60_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_FAIMS_35_45_55_test_C5<-DEB_RNA_FAIMS_40_50_60_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_35_45_

ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction<-C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen
table(ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction)
```

```
##
## False  True
## 20805 15366
```

```
DEB_RNA_FAIMS_40_50_60_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_35_45_55_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_35_45_55_test_C
ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_55_predict
table(ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction_RIPPER)
```

```
##
## False  True
## 17649 18522
```

```
DEB_RNA_FAIMS_40_50_60_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_55_predict
```

Data setup DEB RNA CV 30__50__70

```
DEB_RNA_FAIMS_30_50_70_KNN <- read_delim("AWulf_050919_Ecoli_DEB_S30_FAIMS_30_50_70_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_FAIMS_30_50_70_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_30_50_70_KNN))
names(DEB_RNA_FAIMS_30_50_70_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_30_50_70_KNN))
names(DEB_RNA_FAIMS_30_50_70_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_30_50_70_KNN))
names(DEB_RNA_FAIMS_30_50_70_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_30_50_70_KNN))
DEB_RNA_FAIMS_30_50_70_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_30_50_70_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_30_50_70_KNN$Sample <- "S30"
DEB_RNA_FAIMS_30_50_70_KNN <- DEB_RNA_FAIMS_30_50_70_KNN[DEB_RNA_FAIMS_30_50_70_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_30_50_70_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_30_50_70_KNN$se
DEB_RNA_FAIMS_30_50_70_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_30_50_70_KNN$p
DEB_RNA_FAIMS_30_50_70_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_30_50_70_KNN$prep
DEB_RNA_FAIMS_30_50_70_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_30_50_70_KNN$prepPep)
DEB_RNA_FAIMS_30_50_70_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_30_50_70_KNN$prepPep)
DEB_RNA_FAIMS_30_50_70_KNN$Pepseq <- DEB_RNA_FAIMS_30_50_70_KNN$prepPep
DEB_RNA_FAIMS_30_50_70_KNN$Peplength <- nchar(DEB_RNA_FAIMS_30_50_70_KNN$Pepseq,
    type = "chars")
DEB_RNA_FAIMS_30_50_70_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_30_50_70_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_30_50_70_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_30_50_70_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_30_50_70_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_30_50_70_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_30_50_70_KNN$pI <- round(pI(DEB_RNA_FAIMS_30_50_70_KNN$Pepseq), 2)
DEB_RNA_FAIMS_30_50_70_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_30_50_70_KNN$Pepseq),
    2)
DEB_RNA_FAIMS_30_50_70_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_30_50_70_KNN$RNPxl_best_localizat
DEB_RNA_FAIMS_30_50_70_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_30_50_70_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_30_50_70_KNN$mw <- round(mw(DEB_RNA_FAIMS_30_50_70_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_30_50_70_KNN$cv <- c("35_45")

names(DEB_RNA_FAIMS_30_50_70_KNN)[names(DEB_RNA_FAIMS_30_50_70_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_30_50_70_KNN)[names(DEB_RNA_FAIMS_30_50_70_KNN) == "precursor_error_(_ppm_)"] <- "p
DEB_RNA_FAIMS_30_50_70_KNN <- DEB_RNA_FAIMS_30_50_70_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_30_50_70_KNN_model <- DEB_RNA_FAIMS_30_50_70_KNN %>% select(colnames(ml_DEB_RNA_train_knn
```

Normalizing

```
DEB_RNA_FAIMS_30_50_70_KNN_model_norm <- DEB_RNA_FAIMS_30_50_70_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_30_50_70_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_30_50_70_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_30_50_70_KNN_model_norm[is.na(DEB_RNA_FAIMS_30_50_70_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_30_50_70_KNN_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_30_50_70_KNN_
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_30_50_70_KNN_model$Prediction <- DEB_RNA_FAIMS_30_50_70_KNN_pred
table(DEB_RNA_FAIMS_30_50_70_KNN_model$Prediction)
```

```
##
## False  True
## 27681  3775
```

```
DEB_RNA_FAIMS_30_50_70_KNN$Prediction_KNN <- DEB_RNA_FAIMS_30_50_70_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_FAIMS_35_45_55_test_C5<-DEB_RNA_FAIMS_30_50_70_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_35_45

ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction<-C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen
table(ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction)
```

```
##
## False  True
## 18977 12479
```

```
DEB_RNA_FAIMS_30_50_70_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_35_45_55_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_35_45_55_test_C
ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_55_predict
```

```
table(ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction_RIPPER)
```

```
##
## False  True
## 16185 15271
```

```
DEB_RNA_FAIMS_30_50_70_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_55_predict
```

Data setup DEB RNA CV 45_55_65

```
DEB_RNA_FAIMS_45_55_65_KNN <- read_delim("AWulf_050919_Ecoli_DEB_S30_FAIMS_35_45_55_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_FAIMS_45_55_65_KNN) <- gsub("\\.", "_", names(DEB_RNA_FAIMS_45_55_65_KNN))
names(DEB_RNA_FAIMS_45_55_65_KNN) <- gsub("\\:", "_", names(DEB_RNA_FAIMS_45_55_65_KNN))
names(DEB_RNA_FAIMS_45_55_65_KNN) <- gsub("\\ ", "_", names(DEB_RNA_FAIMS_45_55_65_KNN))
names(DEB_RNA_FAIMS_45_55_65_KNN) <- gsub("\\|", "_", names(DEB_RNA_FAIMS_45_55_65_KNN))
DEB_RNA_FAIMS_45_55_65_KNN$XLinker <- "DEB"
DEB_RNA_FAIMS_45_55_65_KNN$Nucleotides <- "RNA"
DEB_RNA_FAIMS_45_55_65_KNN$Sample <- "S30"
DEB_RNA_FAIMS_45_55_65_KNN <- DEB_RNA_FAIMS_45_55_65_KNN[DEB_RNA_FAIMS_45_55_65_KNN$RNPxl_RNA !=
    "none", ] %>% filter(target_decoy == "target")
DEB_RNA_FAIMS_45_55_65_KNN$prepPep <- gsub("(Carbamyl)", "", as.character(DEB_RNA_FAIMS_45_55_65_KNN$sec
DEB_RNA_FAIMS_45_55_65_KNN$prepPep <- gsub("(Oxidation)", "", as.character(DEB_RNA_FAIMS_45_55_65_KNN$pr
DEB_RNA_FAIMS_45_55_65_KNN$prepPep <- gsub("(Phospho)", "", as.character(DEB_RNA_FAIMS_45_55_65_KNN$prep
DEB_RNA_FAIMS_45_55_65_KNN$prepPep <- gsub("\\(|\\)", "", DEB_RNA_FAIMS_45_55_65_KNN$prepPep)
DEB_RNA_FAIMS_45_55_65_KNN$prepPep <- gsub("\\..*", "", DEB_RNA_FAIMS_45_55_65_KNN$prepPep)
DEB_RNA_FAIMS_45_55_65_KNN$Pepseq <- DEB_RNA_FAIMS_45_55_65_KNN$prepPep
DEB_RNA_FAIMS_45_55_65_KNN$Peplength <- nchar(DEB_RNA_FAIMS_45_55_65_KNN$Pepseq,
    type = "chars")
DEB_RNA_FAIMS_45_55_65_KNN$aa_class_charged <- str_count(DEB_RNA_FAIMS_45_55_65_KNN$Pepseq,
    paste("R|K|D|E", collapse = "|"))
DEB_RNA_FAIMS_45_55_65_KNN$aa_class_polar <- str_count(DEB_RNA_FAIMS_45_55_65_KNN$Pepseq,
    paste("Q|N|H|S|T|Y|C|W", collapse = "|"))
DEB_RNA_FAIMS_45_55_65_KNN$aa_class_hydrophobic <- str_count(DEB_RNA_FAIMS_45_55_65_KNN$Pepseq,
    paste("A|I|L|M|F|V|P|G", collapse = "|"))
DEB_RNA_FAIMS_45_55_65_KNN$pI <- round(pI(DEB_RNA_FAIMS_45_55_65_KNN$Pepseq), 2)
DEB_RNA_FAIMS_45_55_65_KNN$aIndex <- round(aIndex(DEB_RNA_FAIMS_45_55_65_KNN$Pepseq),
    2)
```

```
DEB_RNA_FAIMS_45_55_65_KNN$XL_aa <- gsub("[:A-Z:]", "", DEB_RNA_FAIMS_45_55_65_KNN$RNPxl_best_localizati
DEB_RNA_FAIMS_45_55_65_KNN$Hydro <- round(hydrophobicity(DEB_RNA_FAIMS_45_55_65_KNN$Pepseq,
    scale = "KyteDoolittle"), 2)
DEB_RNA_FAIMS_45_55_65_KNN$mw <- round(mw(DEB_RNA_FAIMS_45_55_65_KNN$Pepseq, monoisotopic = F),
    2)
DEB_RNA_FAIMS_45_55_65_KNN$cv <- c("35_45")

names(DEB_RNA_FAIMS_45_55_65_KNN)[names(DEB_RNA_FAIMS_45_55_65_KNN) == "precursor_m/z"] <- "precursor"
names(DEB_RNA_FAIMS_45_55_65_KNN)[names(DEB_RNA_FAIMS_45_55_65_KNN) == "precursor_error_(_ppm_)"] <- "pr
DEB_RNA_FAIMS_45_55_65_KNN <- DEB_RNA_FAIMS_45_55_65_KNN %>% filter(RNPxl_isPhospho ==
    "0")

DEB_RNA_FAIMS_45_55_65_KNN_model <- DEB_RNA_FAIMS_45_55_65_KNN %>% select(colnames(ml_DEB_RNA_train_knn)
```

Normalizing

```
DEB_RNA_FAIMS_45_55_65_KNN_model_norm <- DEB_RNA_FAIMS_45_55_65_KNN_model %>% select_if(is.numeric)
DEB_RNA_FAIMS_45_55_65_KNN_model_norm <- as.data.frame(lapply(DEB_RNA_FAIMS_45_55_65_KNN_model_norm,
    normalize))
DEB_RNA_FAIMS_45_55_65_KNN_model_norm[is.na(DEB_RNA_FAIMS_45_55_65_KNN_model_norm)] <- 0
```

Classifying DEB RNA FAIMS data

KNN5

```
DEB_RNA_FAIMS_45_55_65_KNN_pred <- knn(train = ml_DEB_RNA_train_knn, test = DEB_RNA_FAIMS_45_55_65_KNN_m
    cl = ml_DEB_RNA_train_knn_labels$Curated, k = 5)
DEB_RNA_FAIMS_45_55_65_KNN_model$Prediction <- DEB_RNA_FAIMS_45_55_65_KNN_pred
table(DEB_RNA_FAIMS_45_55_65_KNN_model$Prediction)
```

```
##
## False  True
## 31915  5024
```

```
DEB_RNA_FAIMS_45_55_65_KNN$Prediction_KNN <- DEB_RNA_FAIMS_45_55_65_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_FAIMS_35_45_55_test_C5<-DEB_RNA_FAIMS_45_55_65_KNN %>%
  select(colnames(ml_DEB_RNA_train))

C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen<-predict(C5_model_DEB_RNA_boost_pen, ml_DEB_RNA_FAIMS_35_45_

ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction<-C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen
table(ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction)
```

```
##
## False  True
## 23372 13567
```

```
DEB_RNA_FAIMS_45_55_65_KNN$Prediction_C5<-C5_DEB_RNA_FAIMS_35_45_55_predict_boost_pen
```

RIPPER

```
RIPPER_DEB_RNA_FAIMS_35_45_55_predict <- predict(RIPPER_model_DEB_RNA, ml_DEB_RNA_FAIMS_35_45_55_test_C5
ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_55_predict
table(ml_DEB_RNA_FAIMS_35_45_55_test_C5$Prediction_RIPPER)
```

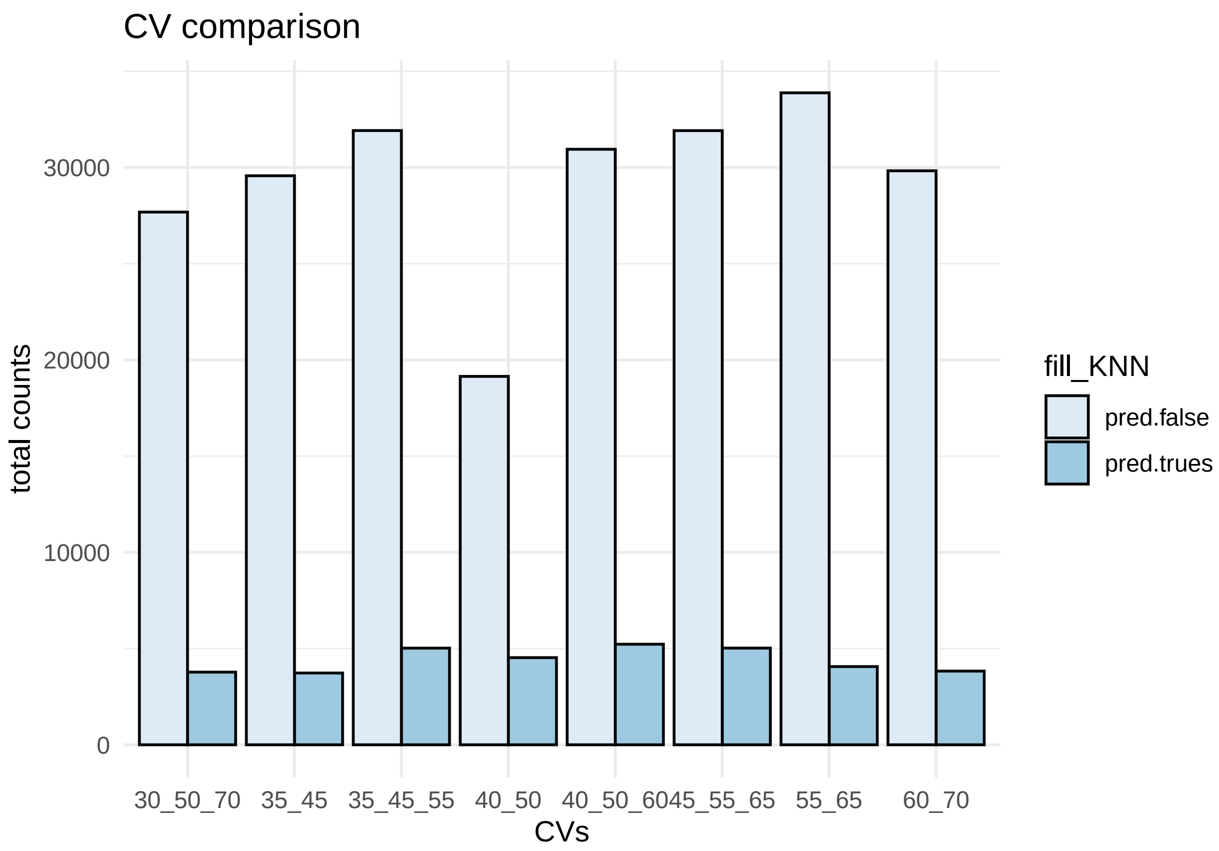
```
##
## False  True
## 20432 16507
```

```
DEB_RNA_FAIMS_45_55_65_KNN$Prediction_RIPPER <- RIPPER_DEB_RNA_FAIMS_35_45_55_predict
```

Comparing the most conservative model KNN

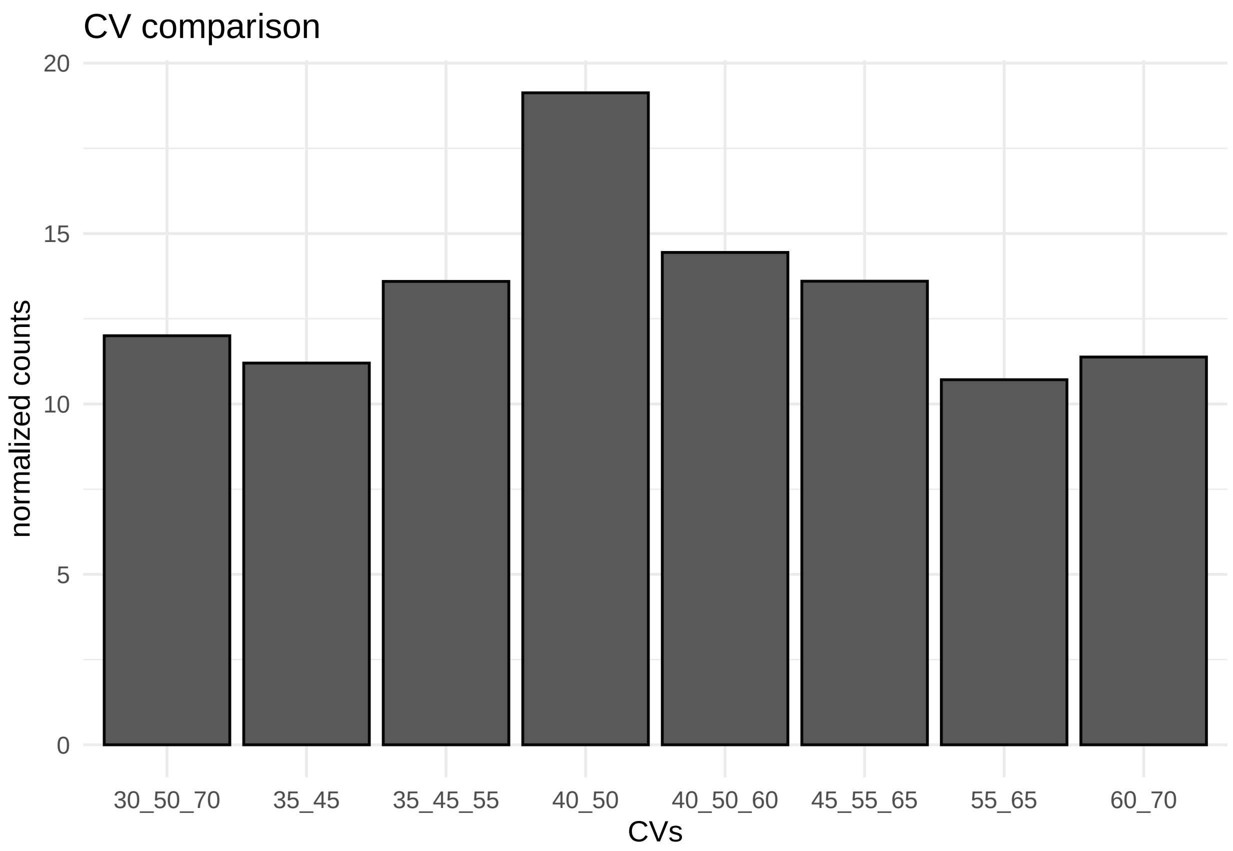
```
a<-as.data.frame(table(DEB_RNA_FAIMS_35_45_KNN$Prediction_KNN))
b<-as.data.frame(table(DEB_RNA_FAIMS_40_50_KNN$Prediction_KNN))
c<-as.data.frame(table(DEB_RNA_FAIMS_55_65_KNN$Prediction_KNN))
d<-as.data.frame(table(DEB_RNA_FAIMS_60_70_KNN$Prediction_KNN))
e<-as.data.frame(table(DEB_RNA_FAIMS_35_45_55_KNN$Prediction_KNN))
f<-as.data.frame(table(DEB_RNA_FAIMS_30_50_70_KNN$Prediction_KNN))
g<-as.data.frame(table(DEB_RNA_FAIMS_40_50_60_KNN$Prediction_KNN))
h<-as.data.frame(table(DEB_RNA_FAIMS_45_55_65_KNN$Prediction_KNN))

f_KNN<-c("double", "double", "double", "double", "double", "double", "double", "double", "double", "dou
x_KNN<-c("35_45", "35_45", "35_45", "40_50","40_50", "40_50", "55_65", "55_65", "55_65", "60_70", "60_7
y_KNN<-c(a[2,2], a[1,2], a[2,2]/(a[2,2]+a[1,2])*100, b[2,2], b[1,2], b[2,2]/(b[2,2]+b[1,2])*100, c[2,2]
fill_KNN<-c("pred.trues", "pred.false", "pred.norm", "pred.trues", "pred.false", "pred.norm", "pred.tru
df_KNN_comparison<-data.frame(x_KNN, y_KNN, fill_KNN, f_KNN)
df_KNN_comparison1<-filter(df_KNN_comparison, fill_KNN!="pred.norm")
df_KNN_comparison2<-filter(df_KNN_comparison, fill_KNN=="pred.norm")
ggplot(df_KNN_comparison1, aes(x=x_KNN, y=y_KNN, fill = fill_KNN))+
  geom_bar(stat = "identity", position=position_dodge(),bindwidth =1, bins = 3, color="gray2")+
  scale_fill_brewer(palette = "Blues")+
  ggtitle("CV comparison")+
  theme_minimal()+
  ylab("total counts")+
  xlab("CVs")
```

```
## Warning: Ignoring unknown parameters: bindwidth, bins
```



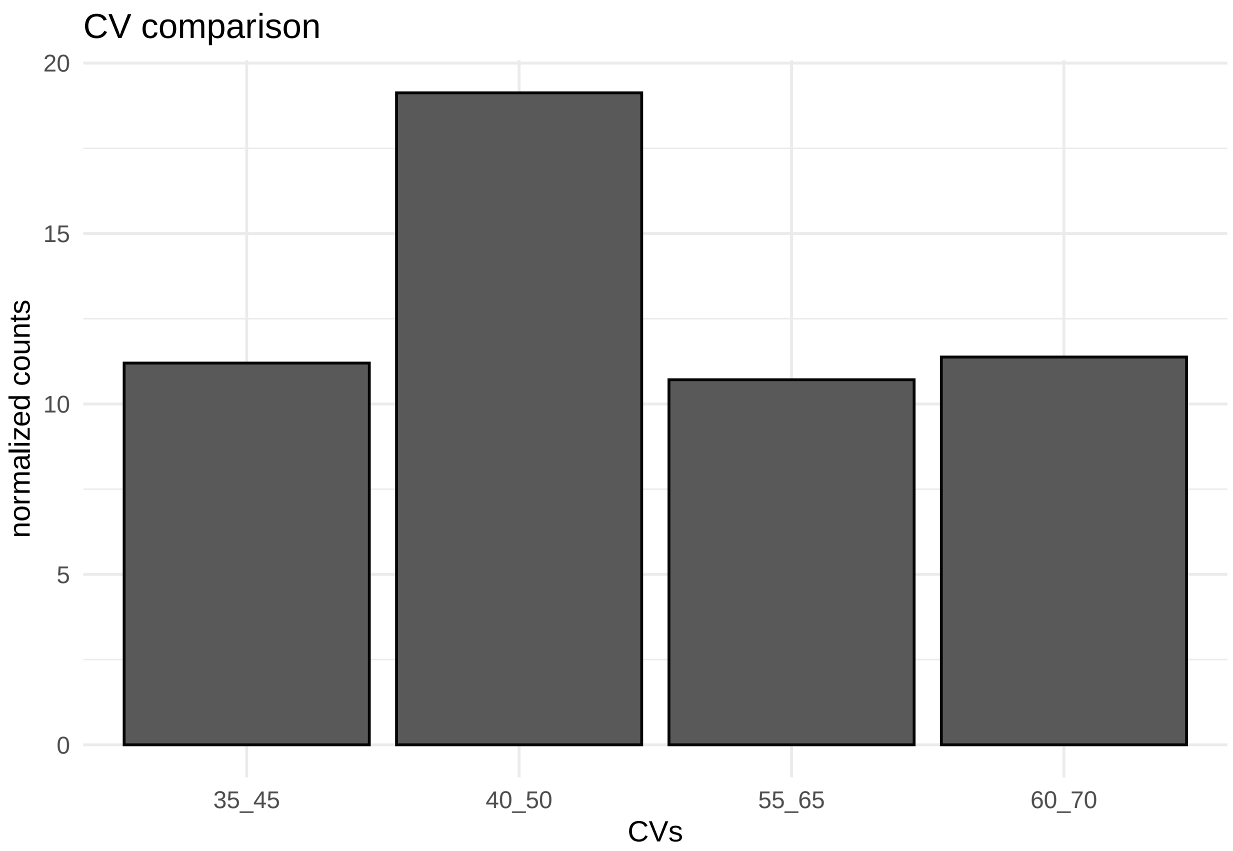
```r
ggplot(df_KNN_comparison2, aes(x=x_KNN, y=y_KNN))+
  geom_bar(stat="identity", bindwidth =1, bins = 3, color="gray2")+
  ggtitle("CV comparison")+
  ylab("normalized counts")+
  xlab("CVs")+
  theme_minimal()
```

```
## Warning: Ignoring unknown parameters: bindwidth, bins
```



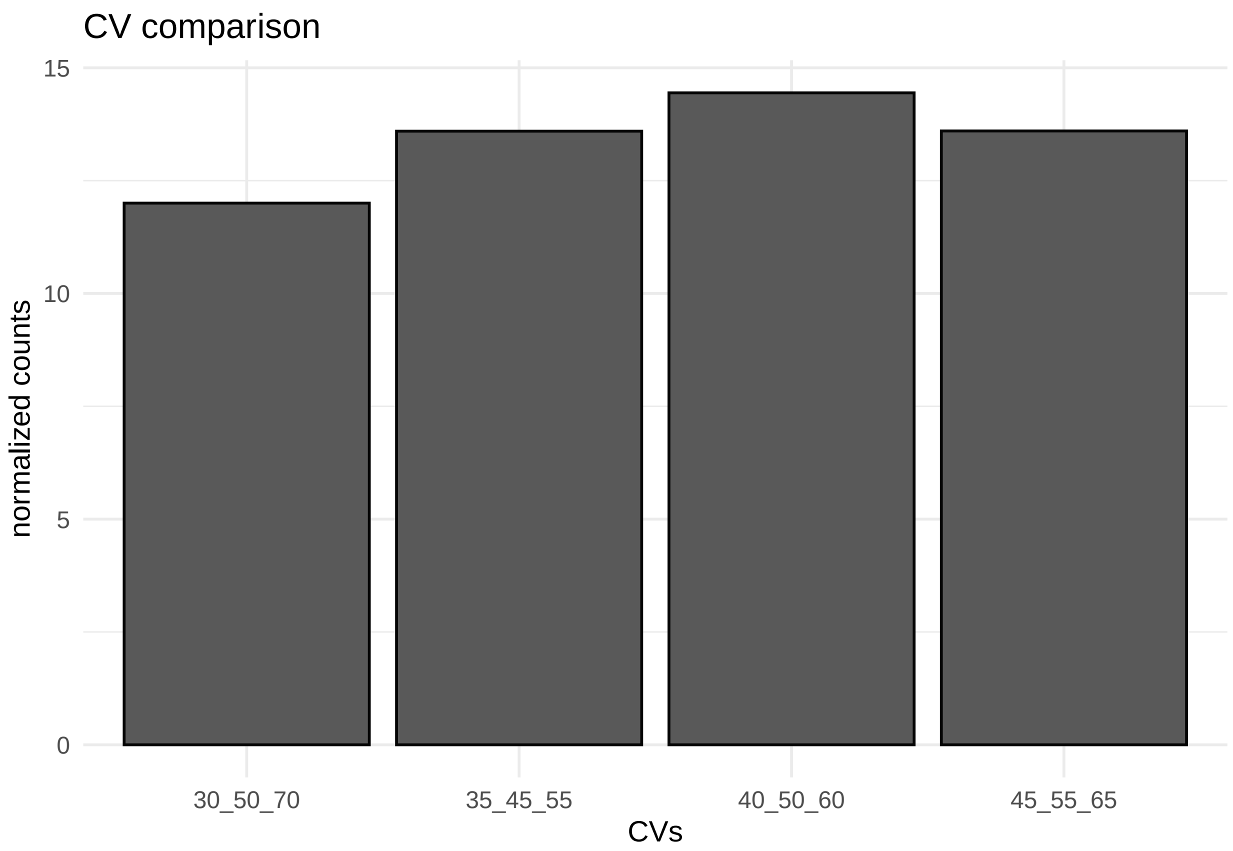
```r
df_KNN_comparison3<-filter(df_KNN_comparison2, f_KNN=="double")

ggplot(df_KNN_comparison3, aes(x=x_KNN, y=y_KNN))+
  geom_bar(stat="identity", bindwidth =1, bins = 3, color="gray2")+
  ggtitle("CV comparison")+
  ylab("normalized counts")+
  xlab("CVs")+
  theme_minimal()
```

```
## Warning: Ignoring unknown parameters: bindwidth, bins
```



```r
df_KNN_comparison4<-filter(df_KNN_comparison2, f_KNN=="triple")

ggplot(df_KNN_comparison4, aes(x=x_KNN, y=y_KNN))+
  geom_bar(stat="identity", bindwidth =1, bins = 3, color="gray2")+
  ggtitle("CV comparison")+
  ylab("normalized counts")+
  xlab("CVs")+
  theme_minimal()
```

```
## Warning: Ignoring unknown parameters: bindwidth, bins
```



Export lists

```
S30_DEB_RNA_FAIMS_total_predicted_trues<- rbind(DEB_RNA_FAIMS_35_45_KNN, DEB_RNA_FAIMS_40_50_KNN, DEB_RN
  filter(Prediction_KNN == "True")

write_excel_csv(S30_DEB_RNA_FAIMS_total_predicted_trues, "S30_DEB_RNA_FAIMS_total_KNN_prediction.csv")
```