3.2-section-result

February 14, 2025

1 Section 3 - Identifying features that may drive outcomes

1.1 Example 3.2

Application 3.2: What features might drive differential translation rates as measured by ribosome profiling when specific amino acids are in the A- and P-sites of the ribosome?

- Translation is a key biological process during which ribosomes synthesize proteins based on messenger RNA (mRNA) templates
- During translation elongation, amino acids are added one at a time to the nascent protein (**Figure 3.2.1**)
- The speed at which amino acids are added can be an important factor in determining if a protein will fold and function or misfold and malfunction

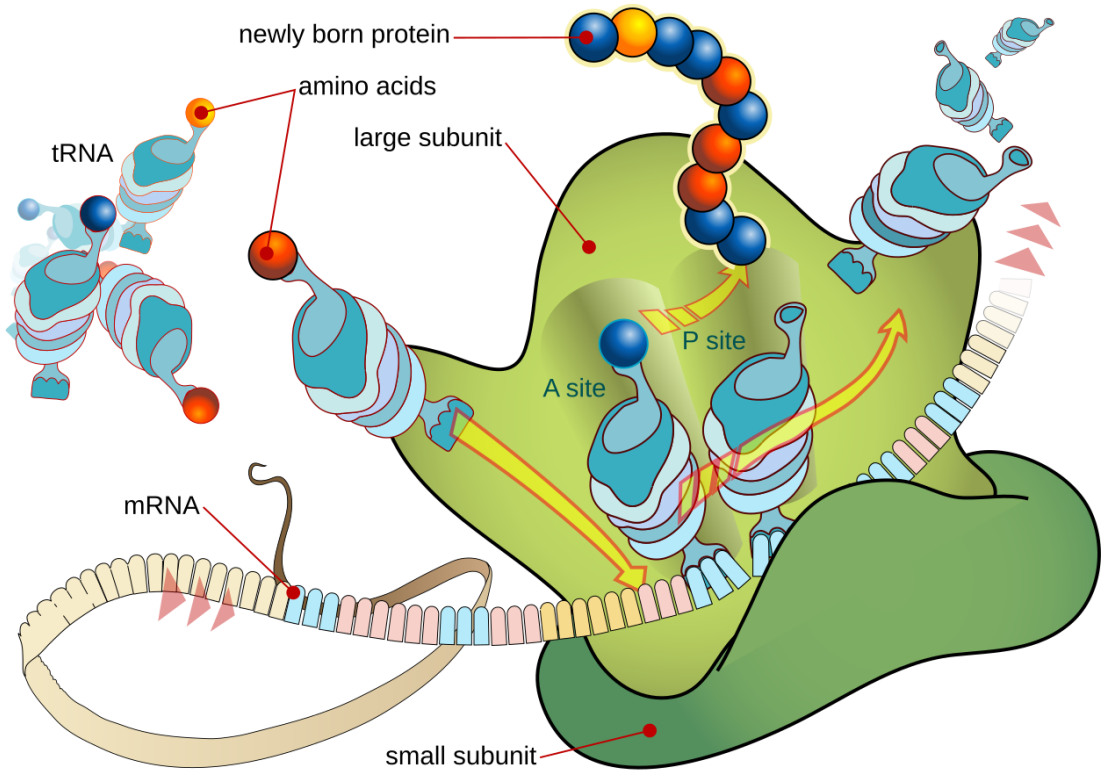


Figure 3.2.1 *The ribosome has three sites that accommodate tRNA: the A-, P- and E-sites. The ribosome ratchets along the mRNA, presenting different mRNA codons for decoding by aminoacyl-tRNA (aa-tRNA) at the A-site and catalyzing peptide bond formation between the nascent protein bound to the*

P-site tRNA and the amino acid bound to the A-site aa-tRNA. The E-site binds the deacylated tRNA before it exits the ribosome. Reproduced from https://en.wikipedia.org/wiki/Translation_(biology)

- Many different factors are thought to influence the speed of translation
- In this application, we will explore using LASSO to determine which physicochemical properties are most useful in predicting how different combinations of amino acids and tRNA in the A- and P-sites of the ribosome influence translation speed

1.1.1 Step 0 - Load libraries

[1]:
```
import pandas as pd
from sklearn.linear_model import LogisticRegression
from sklearn.model_selection import StratifiedKFold, train_test_split,␣
 ↪cross_validate
from sklearn.metrics import roc_auc_score, balanced_accuracy_score
import numpy as np
from datetime import datetime
import matplotlib.pyplot as plt
```

1.1.2 Step 1 - Load the data & explore

- In this instance, the features and outcome are in two separate files

[2]:
```
# data7_features & data7_outcomes are both DataFrame objects
data_path      = "/home/jovyan/data-store/data/iplant/home/shared/NCEMS/
 ↪BPS-training-2025/"
data7_features = pd.read_csv(data_path+"ta_transformed_features.csv")
data7_outcomes = pd.read_csv(data_path+"ta_transformed-data_targets.csv")

# both of these DataFrames have an unneeded first column, so we will remove it
data7_features = data7_features.iloc[:, 1:]
data7_outcomes = data7_outcomes.iloc[:, 1:]

print ("Summary of the features:\n")
display(data7_features.head(10))
display(data7_features.info())

print ("\nSummary of the outcomes:\n")
display(data7_outcomes.head(10))
display(data7_outcomes.info())
```

```
Summary of the features:
```

	molecular-weight	residue-weight	pKa	pKb	pKx	pI	\
0	-1.738756	-1.778904	0.809351	0.511075	0.003250	-0.104711	
1	-0.479951	-0.494600	-1.126355	1.803312	0.003250	-0.579427	
2	-0.005942	-0.010616	-1.531696	0.314324	-2.191927	-1.737091	
3	0.553548	0.562190	0.043208	0.467344	-1.904829	-1.512403	

```
4          1.279681        1.306135 -1.784651 -0.711573  0.003250 -0.370619
5          0.875650        0.891634 -1.835207 -0.624366 -1.061133  0.715915
6          0.515922        0.523672 -0.007773 -1.103767  1.167600  1.844031
7         -0.082676       -0.089064  0.911706  0.314324  0.003250 -0.114960
8          0.636866        0.647490  0.502572 -0.537140  0.003250 -0.237816
9         -0.718141       -0.737814 -0.974158  2.505948  0.003250  0.049249

      hp-kd     bb=sc    charge    volume  …   T-stem2  Acc-stem2       CCA  \
0  1.056406 -0.312761  0.063085 -1.443226  … -0.200366  -0.223654 -0.237966
1  1.474742 -0.312761  0.063085 -0.850264  … -0.200366  -0.223654 -0.237966
2 -1.429836 -0.312761 -3.107030 -0.772395  … -0.200366  -0.223654 -0.237966
3 -1.429836 -0.312761 -3.107030  0.050820  … -0.200366  -0.223654 -0.237966
4  1.662330 -0.312761  0.063085  1.632108  … -0.200366  -0.223654 -0.237966
5 -1.314600 -0.312761  1.064173  0.501420  … -0.200366  -0.223654 -0.237966
6 -1.579684 -0.312761  1.064173  0.973549  … -0.200366  -0.223654 -0.237966
7  2.327081 -0.312761  0.063085  0.915118  … -0.200366  -0.223654 -0.237966
8  1.114574 -0.312761  0.063085  0.798410  … -0.200366  -0.223654 -0.237966
9 -0.655096  3.275925  0.063085 -0.724430  … -0.200366  -0.223654 -0.237966

   Category-Not-Found     Total  AntiCodon3  asitetrna  psitetrna  asiteaa  \
0            1.042375 -0.623204    0.395781       A-t1       A-t1        A
1           -0.766054 -0.190813   -0.765462       A-t1       C-t1        A
2           -0.766054 -1.293589   -0.765462       A-t1       D-t1        A
3           -0.766054 -2.445107   -0.765462       A-t1       E-t2        A
4            0.130562  0.890090    0.395781       A-t1       F-t1        A
5           -0.766054 -0.190813   -0.765462       A-t1       H-t1        A
6            1.042375  0.131705   -0.765462       A-t1       K-t1        A
7            0.130562  1.108903   -0.765462       A-t1       L-t4        A
8            0.130562  0.510462   -0.765462       A-t1       M-t1        A
9            0.130562  0.576202    0.395781       A-t1       P-t1        A

   psiteaa
0        A
1        C
2        D
3        E
4        F
5        H
6        K
7        L
8        M
9        P

[10 rows x 633 columns]

<class 'pandas.core.frame.DataFrame'>
RangeIndex: 808 entries, 0 to 807
Columns: 633 entries, molecular-weight to psiteaa
```

```
dtypes: float64(629), object(4)
memory usage: 3.9+ MB
```

```
None
```

```
Summary of the outcomes:
```

```
  Asite Psite  percent-diff
0  A-t1  A-t1     -8.176901      0
1  A-t1  C-t1    -17.741818      0
2  A-t1  D-t1     16.011557      1
3  A-t1  E-t2    -15.448015      0
4  A-t1  F-t1    -10.063853      0
5  A-t1  H-t1     29.766722      1
6  A-t1  K-t1      5.213059      1
7  A-t1  L-t4      8.685709      1
8  A-t1  M-t1    -16.649137      0
9  A-t1  P-t1     15.481030      1
```

```
<class 'pandas.core.frame.DataFrame'>
RangeIndex: 808 entries, 0 to 807
Data columns (total 4 columns):
 #   Column        Non-Null Count  Dtype
---  ------        --------------  -----
 0   Asite         808 non-null    object
 1   Psite         808 non-null    object
 2   percent-diff  808 non-null    float64
 3            808 non-null    int64
dtypes: float64(1), int64(1), object(2)
memory usage: 25.4+ KB
```

```
None
```

- What we are trying to predict is `Speed`, which is `0` if translation is faster for the given pair of amino acids in the A- and P-sites than average and `1` if it is slower than average
- These values were derived from ribosome profiling, a next-generation sequencing technique that specifically sequences fragments of mRNAs that are covered by the ribosome
- We will attempt to model `Speed` using the set of 629 features in `data7_features`
- Let's make sure that the features have each been scaled correctly

[3]:
```
print ("\nInformation about mean and standard deviation of parameters:")
data7_features.describe()
```

```
Information about mean and standard deviation of parameters:
```

[3]:
```
       molecular-weight  residue-weight           pKa           pKb  \
count        808.000000    8.080000e+02  8.080000e+02  8.080000e+02
mean           0.000000   -1.758769e-17 -6.595384e-18  1.538923e-17
```

```
std            1.000619    1.000619e+00  1.000619e+00  1.000619e+00
min           -2.442131   -2.527703e+00 -2.064775e+00 -1.477948e+00
25%           -0.591934   -5.989729e-01 -4.833735e-01 -7.318487e-01
50%           -0.036196   -3.525555e-02  4.335576e-02 -1.464536e-01
75%            0.528912    5.296224e-01  7.321650e-01  3.600857e-01
max            3.054014    3.167796e+00  3.771015e+00  2.632413e+00

                pKx            pI         hp-kd         bb=sc        charge  \
count  8.080000e+02  8.080000e+02  8.080000e+02  8.080000e+02  8.080000e+02
mean  -6.155692e-17  5.056461e-17  8.793846e-18  3.077846e-17  8.793846e-18
std    1.000619e+00  1.000619e+00  1.000619e+00  1.000619e+00  1.000619e+00
min   -3.003972e+00 -2.329694e+00 -2.728074e+00 -3.127613e-01 -3.107030e+00
25%   -2.127222e-03 -2.904044e-01 -6.344190e-01 -3.127613e-01  6.308469e-02
50%    3.250223e-03 -1.261844e-01 -9.895475e-02 -3.127613e-01  6.308469e-02
75%    3.250223e-03  1.075296e-02  4.129708e-01 -3.127613e-01  3.800961e-01
max    3.012865e+00  3.323830e+00  3.546362e+00  3.275925e+00  1.064173e+00

              volume  …      Ac-stem2      V-region       T-stem1  \
count  8.080000e+02  …  8.080000e+02  8.080000e+02  8.080000e+02
mean   1.703808e-17  …  9.893076e-18  4.726692e-17  1.099231e-17
std    1.000619e+00  …  1.000619e+00  1.000619e+00  1.000619e+00
min   -2.618727e+00  … -8.503111e-01 -1.985877e+00 -8.393183e-01
25%   -6.601098e-01  … -8.503111e-01 -5.922514e-01 -8.393183e-01
50%    9.945293e-02  … -2.269404e-01 -1.071175e-01 -3.220408e-01
75%    7.649899e-01  …  4.185946e-01  5.382254e-01  1.605993e+00
max    3.208572e+00  …  3.830079e+00  2.849427e+00  1.605993e+00

              T-loop       T-stem2     Acc-stem2           CCA  \
count  8.080000e+02  8.080000e+02  8.080000e+02  8.080000e+02
mean   1.868692e-17  1.099231e-16  1.758769e-17  2.462277e-16
std    1.000619e+00  1.000619e+00  1.000619e+00  1.000619e+00
min   -1.370088e+00 -2.003655e-01 -2.236536e-01 -2.379662e-01
25%   -1.114230e+00 -2.003655e-01 -2.236536e-01 -2.379662e-01
50%    7.649454e-01 -2.003655e-01 -2.236536e-01 -2.379662e-01
75%    7.649454e-01 -2.003655e-01 -2.236536e-01 -2.379662e-01
max    9.140131e-01  5.769773e+00  4.628624e+00  4.675962e+00

       Category-Not-Found         Total    AntiCodon3
count        8.080000e+02  8.080000e+02  8.080000e+02
mean        -3.077846e-17  8.793846e-18  5.496154e-18
std          1.000619e+00  1.000619e+00  1.000619e+00
min         -2.515191e+00 -3.154981e+00 -1.892550e+00
25%          1.305618e-01 -4.721559e-01 -7.654615e-01
50%          1.417253e-01  1.791005e-01  3.957811e-01
75%          1.417253e-01  6.095507e-01  3.957811e-01
max          3.008398e+00  2.722634e+00  4.148644e+00
```

```
[8 rows x 629 columns]
```

- We can see that the features have already been scaled - they have a mean of zero and standard deviation of one
- Finally, we need to check the balance of the outcome classes

[4]:
```
# calculate counts per outcome class
class_counts = data7_outcomes["Speed"].value_counts()
print (class_counts)
```

```
Speed
0    439
1    369
Name: count, dtype: int64
```

- We can see that the `0` class accounts for 54% of that data and the `1` class accounts for 46% of the data
- This is reasonably well balanced, but we should remain aware of the class imbalance all the same

1.1.3 Step 2 - Prepare data for model building

- As in **Example 3.1**, we will use k-fold cross-validation with a grid search over

[5]:
```
# set random seed to achieve reproducible results
random_seed = 1

# number of folds for cross-validation
Nfolds      = 5

# define feature and outcome data sets; note that we need to drop some␣
 ↪non-numerical columns from the feature space
X           = data7_features.drop(["asitetrna", "psitetrna", "asiteaa",␣
 ↪"psiteaa"], axis=1)
y           = data7_outcomes["Speed"]

# reserve 20% of data for final testing after hyperparameter tuning
X_train, X_holdout, y_train, y_holdout = train_test_split(X, y, test_size=0.2,␣
 ↪random_state=random_seed, stratify=y)

# set up k-fold cross-validation with outcome stratification
kf          = StratifiedKFold(n_splits=Nfolds, shuffle=True,␣
 ↪random_state=random_seed)

# define a range of lambda values to be used in our grid search
lambda_vals = np.logspace(-1, 4, 6)
```

1.1.4 Step 3 - Optimize

- We are now ready to run cross-validation for each value of and decide which value we want to use for our final model

[6]:
```
# record the start time
startTime    = datetime.now()

# maximum number of iterations to be run
max_iter     = 20000

# setup dictionary to store results for each value of lambda
results_dict = {}

# loop over lambda values
for lambda_val in lambda_vals:

    # setup logistic regression model
    model                    = LogisticRegression(penalty="l1", solver="saga",
                                                  max_iter=max_iter, C=1/
 ↪lambda_val)

    # run cross-validation for current lambda_val
    cv_results               = cross_validate(model, X_train, y_train, cv=kf,␣
 ↪return_estimator=True,
                                              scoring=['balanced_accuracy',␣
 ↪'roc_auc'], n_jobs=-1)

    # store results for later
    results_dict[lambda_val] = cv_results

    # calculation elapsed time and print it to the screen
    elapsed_sec              = (datetime.now() - startTime).total_seconds()
    print(f"{lambda_val:10.4f} {elapsed_sec:10.2f} s")
```

```
    0.1000      28.74 s
    1.0000      47.59 s
   10.0000      61.83 s
  100.0000      63.31 s
 1000.0000      63.52 s
10000.0000      63.72 s
```

- Let's assess performance and number of features as a function of

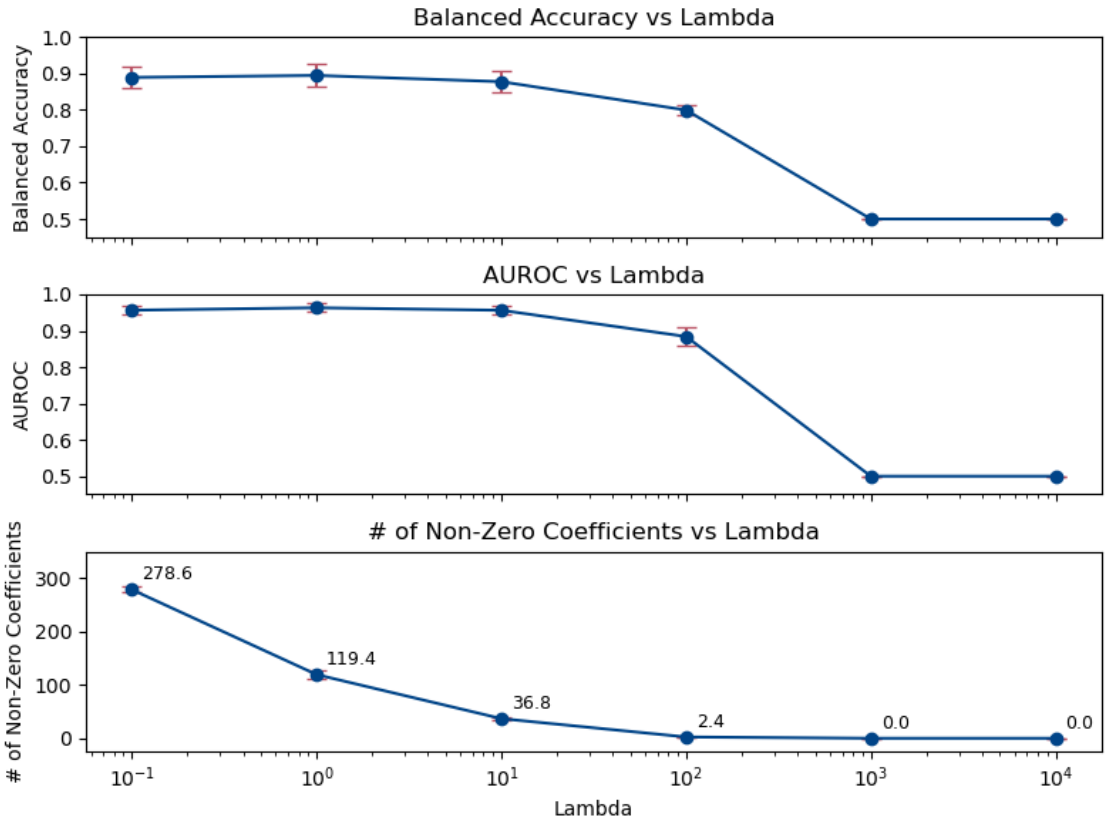
[7]:
```
# sort the lambda values
lambda_vals = sorted(results_dict.keys())

# initialize lists to store the aggregated metric means and standard deviations
bal_acc_means, bal_acc_stds = [],[]
```

```
auroc_means, auroc_stds     = [],[]
nonzero_means, nonzero_stds = [],[]

# loop over each lambda and compute metrics
for lambda_val in lambda_vals:

    cv_results     = results_dict[lambda_val]

    # extract balanced accuracy and AUROC scores
    test_bal_acc   = cv_results['test_balanced_accuracy']
    test_roc_auc   = cv_results['test_roc_auc']

    # compute mean and standard deviation
    mean_bal_acc   = np.mean(test_bal_acc)
    std_bal_acc    = np.std(test_bal_acc, ddof=1)
    mean_roc_auc   = np.mean(test_roc_auc)
    std_roc_auc    = np.std(test_roc_auc, ddof=1)

    # compute number of non-zero coefficients for each fold
    nonzero_counts = [np.count_nonzero(estimator.coef_[0]) for estimator in␣
 ↪cv_results['estimator']]
    mean_nonzero   = np.mean(nonzero_counts)
    std_nonzero    = np.std(nonzero_counts, ddof=1)

    # Append the computed metrics to the corresponding lists
    bal_acc_means.append(mean_bal_acc)
    bal_acc_stds.append(std_bal_acc)
    auroc_means.append(mean_roc_auc)
    auroc_stds.append(std_roc_auc)
    nonzero_means.append(mean_nonzero)
    nonzero_stds.append(std_nonzero)

# print summary information to screen
header = ("Lambda".ljust(12) + "Balanced Acc (mean ± std)".ljust(27) +
          "AUROC (mean ± std)".ljust(30) + "Non-zero Coeffs (mean ± std)")
print(header)

for i, lambda_val in enumerate(lambda_vals):
    nonzero_str = f"{nonzero_means[i]:10.1f} ± {nonzero_stds[i]:10.1f}"
    print(f"{lambda_val:10.4f}\t" f"{bal_acc_means[i]:0.3f} ± {bal_acc_stds[i]:
 ↪0.3f}\t\t"
          f"{auroc_means[i]:0.3f} ± {auroc_stds[i]:0.3f}\t\t" f"{nonzero_str}")

# create summary plots
plot_color  = "#004488"
error_color = "#BB5566"
fig, axes   = plt.subplots(3, 1, figsize=(8, 6), sharex=True)
```

```
# plot Balanced Accuracy
axes[0].errorbar(lambda_vals, bal_acc_means, yerr=bal_acc_stds, fmt='o-',␣
 ↪capsize=5, color=plot_color, ecolor=error_color)
axes[0].set_xscale('log')
axes[0].set_ylabel('Balanced Accuracy')
axes[0].set_ylim(0.45, 1.0)
axes[0].set_yticks([0.5, 0.6, 0.7, 0.8, 0.9, 1.0])
axes[0].set_title('Balanced Accuracy vs Lambda')

# plot AUROC
axes[1].errorbar(lambda_vals, auroc_means, yerr=auroc_stds, fmt='o-',␣
 ↪capsize=5, color=plot_color, ecolor=error_color)
axes[1].set_xscale('log')
axes[1].set_ylabel('AUROC')
axes[1].set_ylim(0.45, 1.0)
axes[1].set_yticks([0.5, 0.6, 0.7, 0.8, 0.9, 1.0])
axes[1].set_title('AUROC vs Lambda')

# plot number of non-zero coefficients
axes[2].errorbar(lambda_vals, nonzero_means, yerr=nonzero_stds, fmt='o-',␣
 ↪capsize=5,color=plot_color, ecolor=error_color)
axes[2].set_xscale('log')
axes[2].set_ylabel('# of Non-Zero Coefficients')
axes[2].set_title('# of Non-Zero Coefficients vs Lambda')
axes[2].set_ylim(-25, 350)
axes[2].set_xlabel('Lambda')

# annotate each point with the mean number of non-zero coefficients (to one␣
 ↪decimal)
for i, lambda_val in enumerate(lambda_vals):
    axes[2].annotate(f"{nonzero_means[i]:.1f}", (lambda_val, nonzero_means[i]),
                     textcoords="offset points", xytext=(5, 5), fontsize=9,␣
 ↪color='black')

plt.tight_layout()
plt.show()
```

```
Lambda      Balanced Acc (mean ± std)  AUROC (mean ± std)            Non-zero
Coeffs (mean ± std)
    0.1000      0.889 ± 0.030           0.957 ± 0.013               278.6 ±
5.5
    1.0000      0.895 ± 0.031           0.963 ± 0.011               119.4 ±
6.8
   10.0000      0.878 ± 0.029           0.956 ± 0.011                36.8 ±
1.6
  100.0000      0.799 ± 0.014           0.884 ± 0.024                 2.4 ±
```

```
0.5
 1000.0000       0.500 ± 0.000              0.500 ± 0.000                    0.0 ±
0.0
10000.0000       0.500 ± 0.000              0.500 ± 0.000                    0.0 ±
0.0
```



- This plot and the associated data indicate that = 1 provides the best model performance based on balanced accuracy & AUROC
- As in **Exercise 3.1**, we will use a larger value of = 10 to limit the size of the non-zero feature space, improving interpretability

1.1.5 Step 4 - Build & test the final model

[8]:
```
# choose our preferred value of lambda
final_lambda        = 10.

# setup the model
final_model         = LogisticRegression(penalty="l1", solver="saga",␣
 ↪max_iter=max_iter, C=1/final_lambda)

# fit the model to the data
```

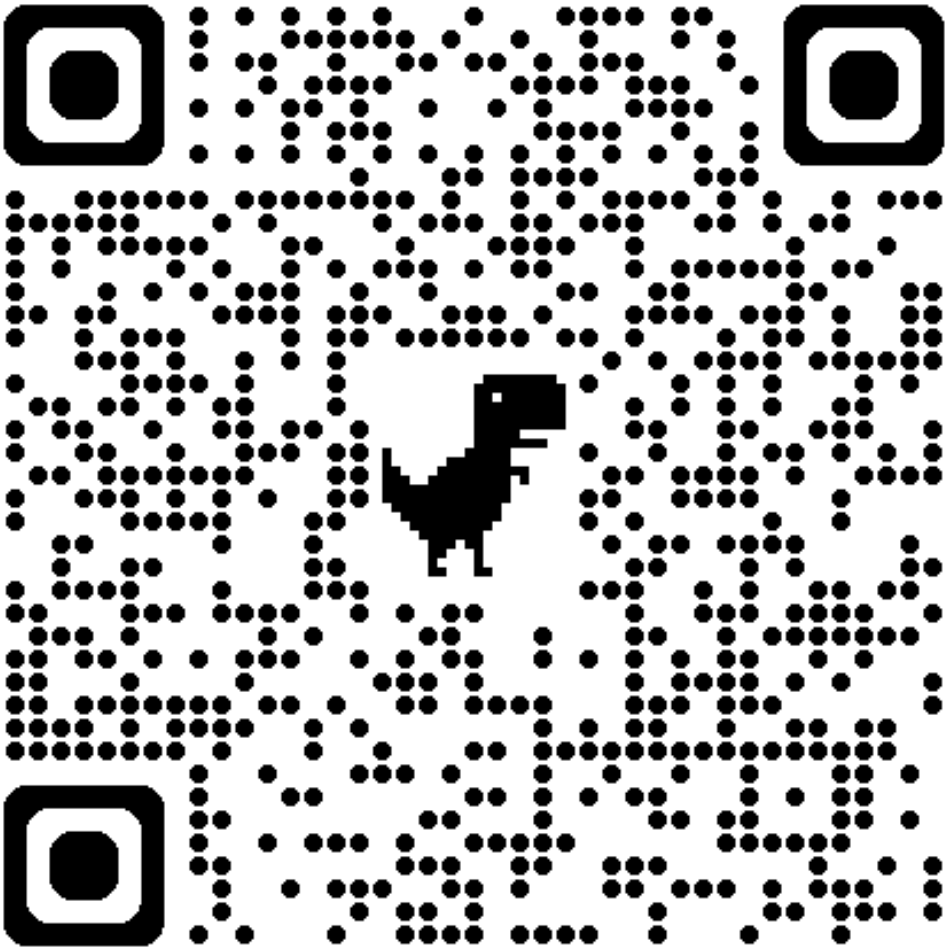
```
final_model.fit(X_train, y_train)

# evaluate the final model on the holdout dataset
y_holdout_pred_prob = final_model.predict_proba(X_holdout)[:, 1]
y_holdout_pred      = final_model.predict(X_holdout)
holdout_auroc       = roc_auc_score(y_holdout, y_holdout_pred_prob)
holdout_bal_acc     = balanced_accuracy_score(y_holdout, y_holdout_pred)

print ("Performance on holdout data\n")
print("Holdout AUROC             :", '%.3f' %holdout_auroc)
print("Holdout Balanced Accuracy :", '%.3f' %holdout_bal_acc, "\n")

# extract the nonzero coefficients
coef                = final_model.coef_.flatten()
nonzero_indices     = coef != 0
nonzero_coefs       = coef[nonzero_indices]
nonzero_features    = X_train.columns[nonzero_indices]

# sort coefficients by absolute magnitude in descending order
sorted_indices      = abs(nonzero_coefs).argsort()[::-1]
sorted_features     = nonzero_features[sorted_indices]
sorted_coefs        = nonzero_coefs[sorted_indices]

# print nonzero coefficients
print(len(sorted_coefs), "Nonzero Coefficients (sorted by magnitude)\n")
for feature, value in zip(sorted_features, sorted_coefs):
    print(feature.ljust(26) + ": " + "%.5f" % value)
```

```
Performance on holdout data

Holdout AUROC             : 0.935
Holdout Balanced Accuracy : 0.849

41 Nonzero Coefficients (sorted by magnitude)

V-region                  : -0.82448
2NT-Codon-Anticodon       : 0.80059
CHOP780207                : 0.55514
3                         : -0.37403
RICJ880116                : 0.37261
MAXF760103                : 0.36795
VASM830101                : 0.35591
+                         : -0.34916
I                         : -0.25216
X                         : 0.24667
.                         : 0.24667
AURR980111                : -0.24317
```

```
Ac-loop                 : 0.22366
solubility              : 0.21016
SUEM840101              : -0.19239
"                       : 0.16712
&                       : 0.14531
GEIM800104              : -0.13244
QIAN880122              : 0.13211
MAXF760101              : -0.12514
MEEJ810101              : -0.12171
RADA880102              : -0.11207
ROBB760113              : 0.11034
QIAN880124              : 0.09961
CHOP780201              : -0.08856
1                       : -0.08205
T-loop                  : 0.07790
ROBB760102              : -0.07715
GUYH850105              : -0.07701
AURR980106              : -0.06880
CHOP780212              : 0.06243
KUMS000103              : -0.05346
R                       : 0.03707
D-stem1                 : 0.03177
NADH010106              : 0.02161
CORJ870105              : 0.01851
concentration           : -0.01276
TANS770108              : 0.01103
WILM950101              : -0.00685
BIOV880102              : 0.00608
J                       : -0.00031
```

1.1.6 Step 5 - Assess the results

- When you are done, consider the following questions:
  - If you were tasked with using the results of this analysis to formulate a hypothesis about the features that are critical for determining translation speed, where would you start?
  - If we were satisfied with lower accuracy but wanted a smaller number of features to interpret, how could we achieve this?
- Use the QR code below to test your knowledge



Quiz Link