Data294P Executive Level Presentation

AN DL APPROACH ON PEAK RECOGNITION IN DNA SEQUENCE

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Background Introduction

This project aims to identify peaks (high accessibility fragments on a DNA sequence), which are associated with condensate formation within Liquid-Liquid Phase Separation (LLPS), through a direct deep learning approach to learn the pattern of the original DNA sequence (the ATCGs).

The overall idea is to do a two-step approach:

- Step 1: generate a label for each peak as yes/no to being a target peak
- Step 2: feed the DNA sequences of the peaks together with the labels generated in step 1 to a deep neural network and train a model

By successfully building this model, we would have much greater efficiency in identifying these peaks with biological significance. It would save us time, as well as cut short the expense conducting formal experiments.

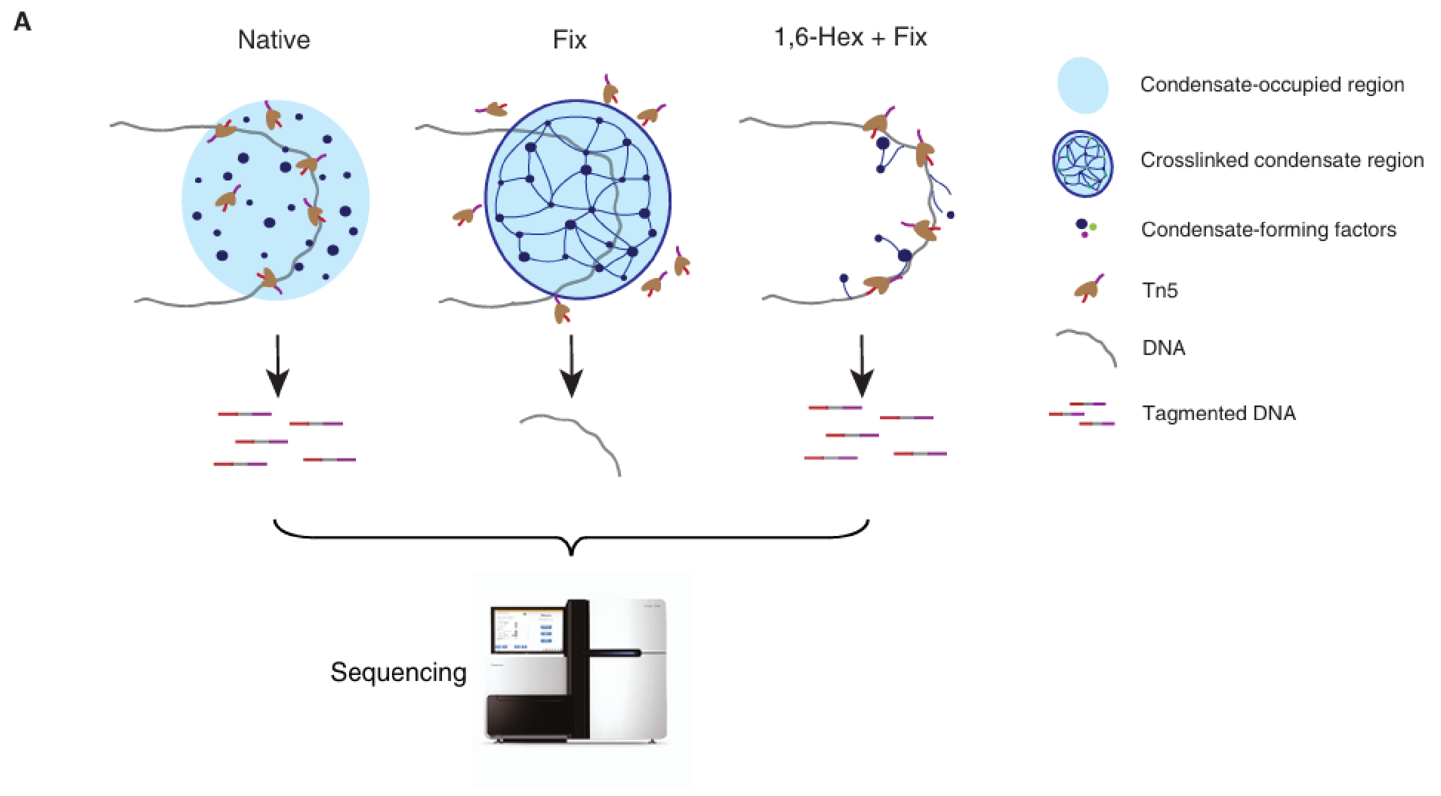
Background Introduction

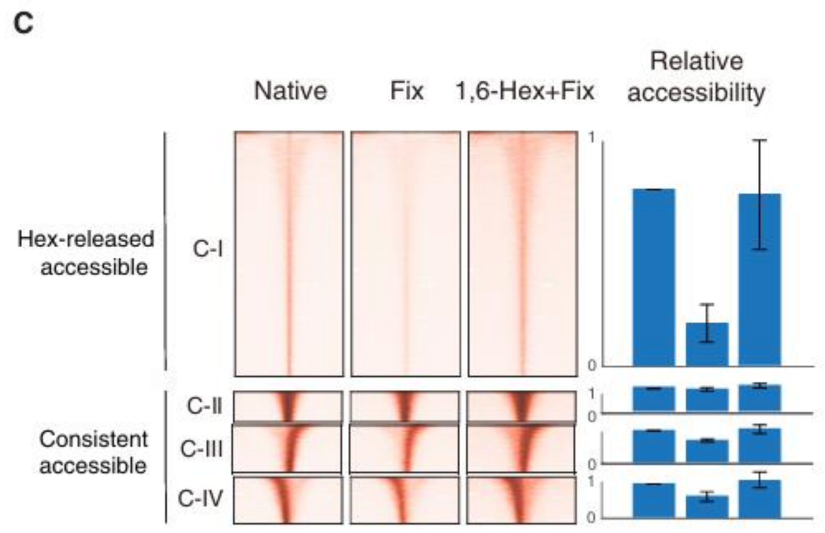
One important feature of the peaks we are looking for is that their responses under a specific set of experimental conditions show **a certain pattern**.

For the three experimental conditions, namely native condition (the control group), chemical fixation (Fix group), and chemical fixation with 1,6-hexanediol (HexFix group), **the relative accessibility (which comes in a positive real number) is significantly lower for the Fix group comparing to the other two groups**, between which no significant difference is observed.

This is because under chemical fixation, the transcriptional components form multivalent, dynamic, intermolecular inter actions that are sensitive to disruption by 1,6-hexanediol(1).

In conclusion, we are looking for a **V-shape** pattern across the 3 experimental conditions.


A
Native
Fix
1,6-Hex + Fix
Condensate-occupied region
Crosslinked condensate region
Condensate-forming factors
Tn5
DNA
Tagmented DNA
Sequencing


C
Native
Fix
1,6-Hex+Fix
Relative accessibility
Hex-released accessible
C-I
Consistent accessible
C-II
C-III
C-IV
1
0

ATAC-seq Technique

The ATAC-seq technique enables the genome-wide detection of chromatin accessibility and plays a crucial role in various fields such as gene regulation and disease mechanisms.

For an individual experimental treatment, raw data produced by ATAC-seq can be converted into a vector using some bioinformatics software. The dimension of the vector is the number of **open regions or peaks**, with each component representing a peak and its value indicating the **relative quantification** of accessibility of that peak.

Proper Normalization

By relative quantification, it means that the accessibility values being recorded are relative to other values within the same treatment group. For example, in a certain experimental group, peak A and peak B having accessibilities of 1 and 2 is no different from them having accessibilities of 100 and 200.

If peak A has accessibility 1, 10, and 100 across the Fresh, Fix, and HexFix groups, we cannot directly compare these three numbers, for they are all “relative” to other accessibility values in their specific group. For example, 1 could be the maximum value in Fresh group, while 100 could be the minimum value in HexFix group, then it will be wrong to conclude that 100 is “larger” than 1.

To determine the “pattern” of a peak is to compare the accessibilities across different experimental groups, thus a normalization between groups is necessary before the comparison.

Data Summary

The dataset is consist of 2 subsets, one of which will be used to make labels, the other to train the model.

The first subset only has 1 table, which has 68809 rows, each row represents a peak of length 400 and has 6 columns:

- chrom: the name of the chrome the peak is on
- start: the start point of the peak
- end: the end point of the peak
- Fresh: the accessibility read for the control group
- Fix: the accessibility read under chemical fixation
- HexFix: the accessibility read under chemical fixation with 1,6-hexanediol

The second subset has 2 tables, each has 137923 rows; each row represents a peak of length 600. The first table in this subset has the following columns:

- chrom: the name of the chrome the peak is on
- start: the start point of the peak
- end: the end point of the peak
- id: the name of peak
- signal: a signal strength calculated by a bio-info specific software when generating the sequence
- label: labels generated from the first step
- intensity: the average intensity for the sequence (the true intensity of a sequence should be a distribution with one read for each nucleotide, but here I just get the average over the distribution)

The second table has the following columns:

- id: the name of the peak
- sequence: the actual sequence (consist of A, T, C, G, and N) of the peak, length is 600

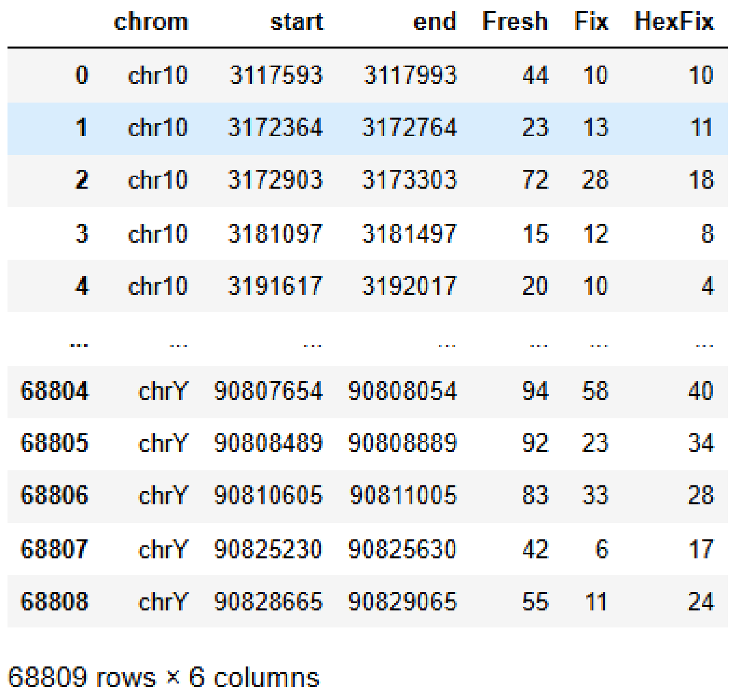
Note that the peaks of the two subsets are not of a 1-to-1 relationship. The peaks in subset 1 underwent a special treatment called overlapping (which is not done by me), and as a result, peaks in subset 1 could correspond to one or several peaks in subset 2, while some peaks in subset 2 are not included in subset 1 at all.

Project Outline

- Data normalization
- Data transformation
- Clustering algorithm (Kmeans) to generate label
- Data cleaning
- One-hot encoding DNA sequence
- Train a CNN model using the one-hot encoded sequence and generated label
- Discussion

Data Normalization

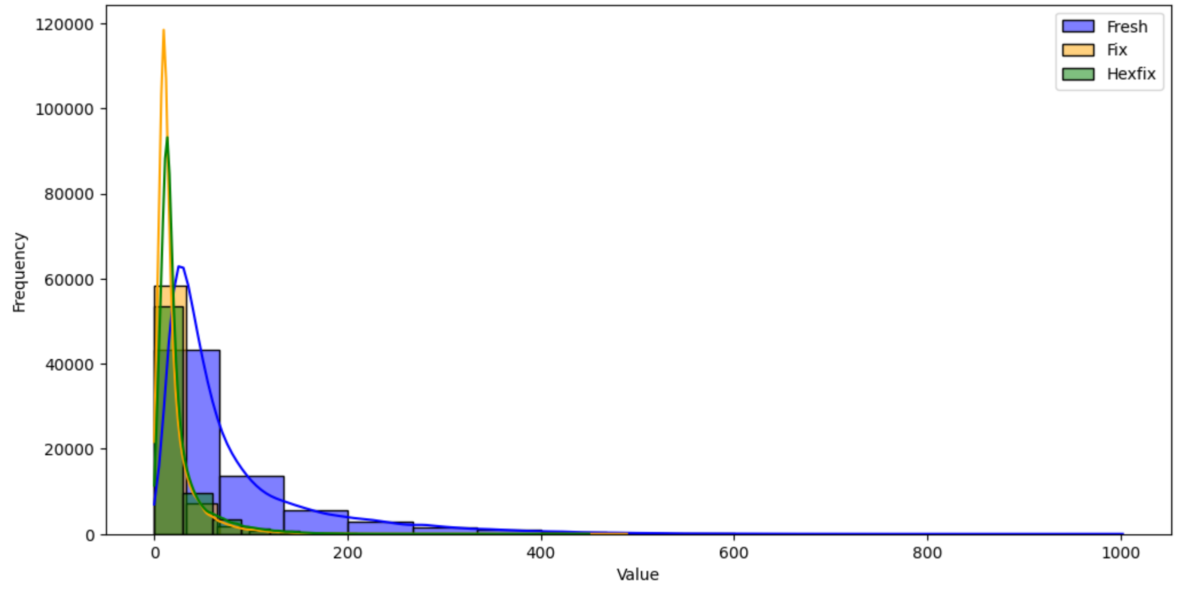
As mentioned before, proper normalization is necessary before comparison between groups. But even before that, let us take a closer look at our data:

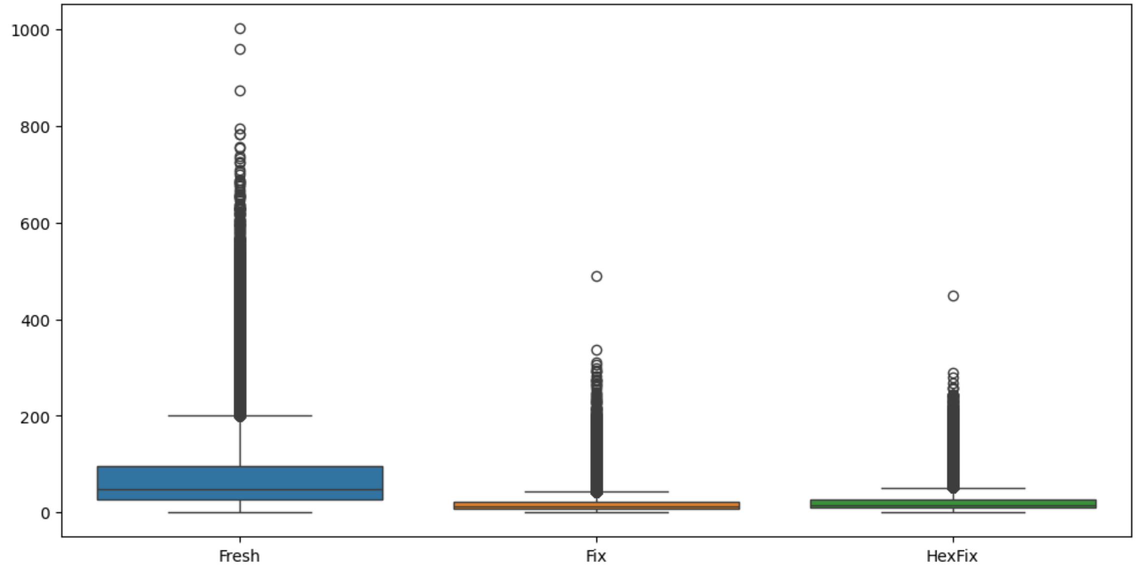


	chrom	start	end	Fresh	Fix	HexFix
0	chr10	3117593	3117993	44	10	10
1	chr10	3172364	3172764	23	13	11
2	chr10	3172903	3173303	72	28	18
3	chr10	3181097	3181497	15	12	8
4	chr10	3191617	3192017	20	10	4
...	...	...	...	...	...	...
68804	chrY	90807654	90808054	94	58	40
68805	chrY	90808489	90808889	92	23	34
68806	chrY	90810605	90811005	83	33	28
68807	chrY	90825230	90825630	42	6	17
68808	chrY	90828665	90829065	55	11	24

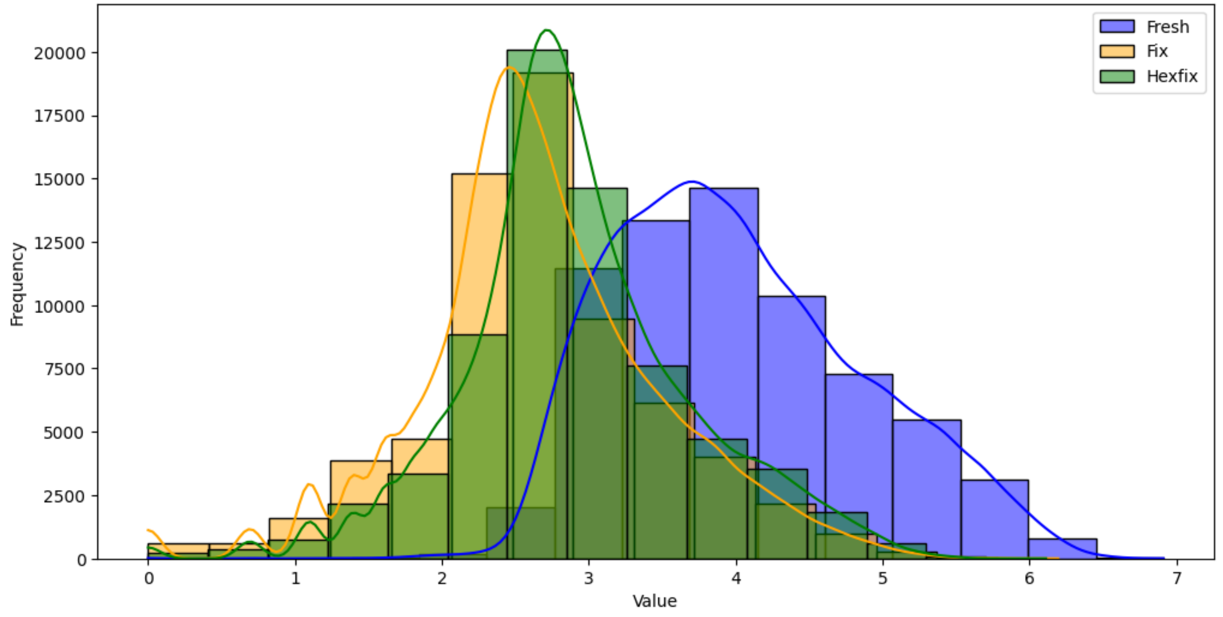
68809 rows × 6 columns

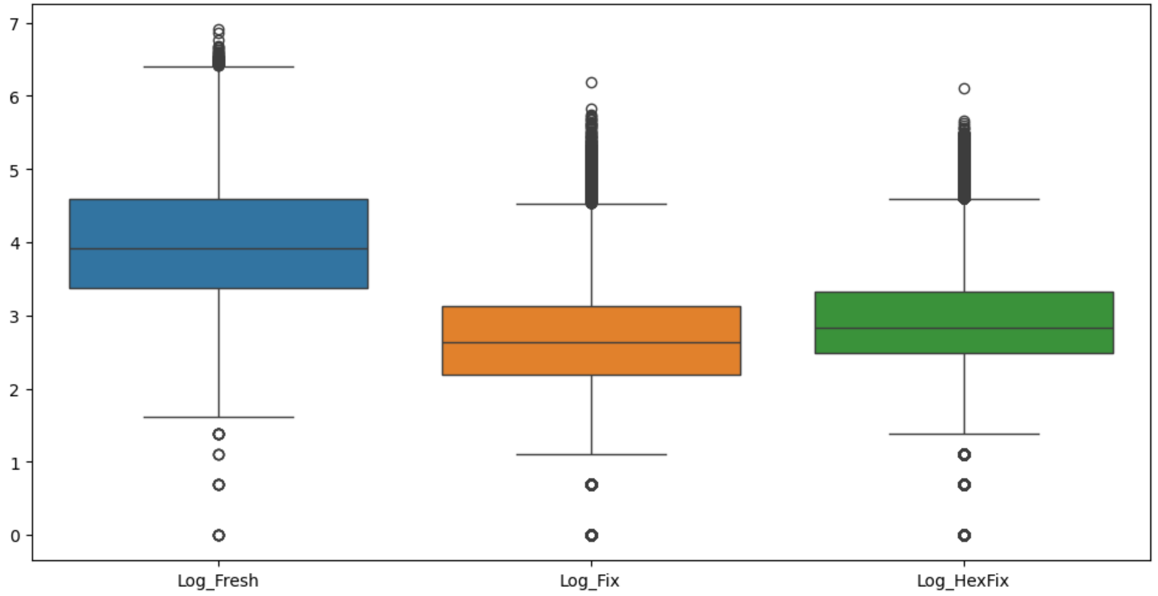
From both the histogram and the box plot we can see that the data is very skewed to the right, and also the three columns are of different “scale”.



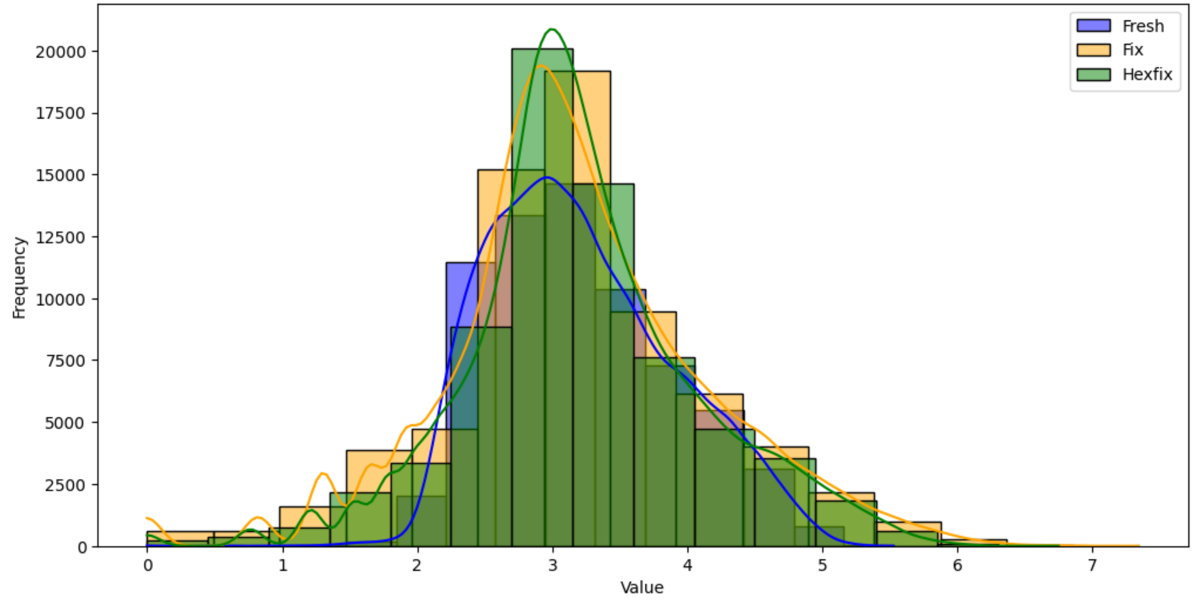


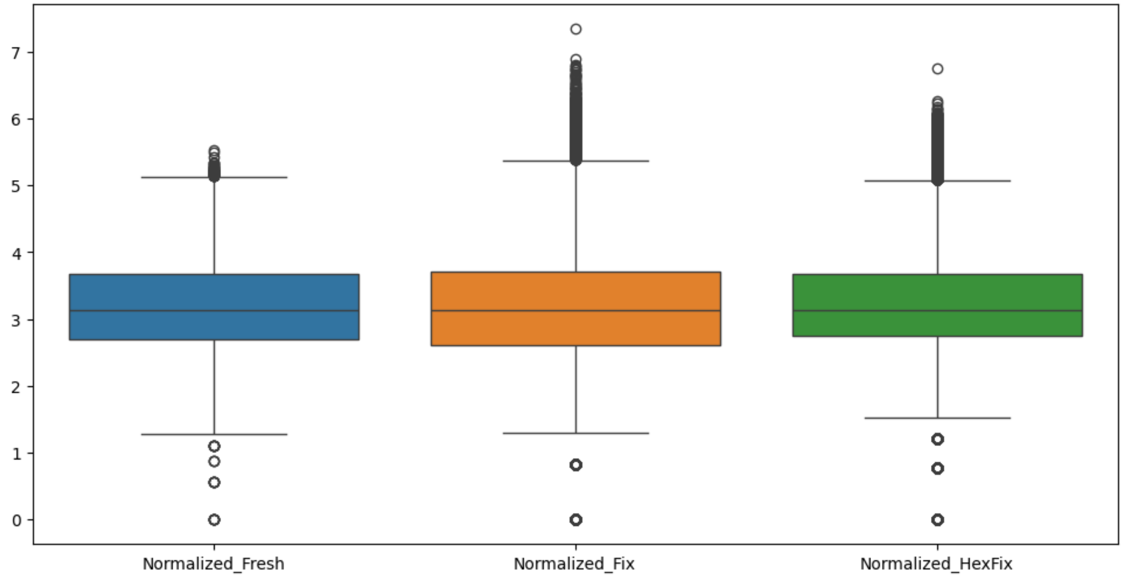
One common approach to deal with data with a long tail is to apply a log transformation. Here, a log2 transformation is used. Can see the data become more "normal" after applying the log transformation.



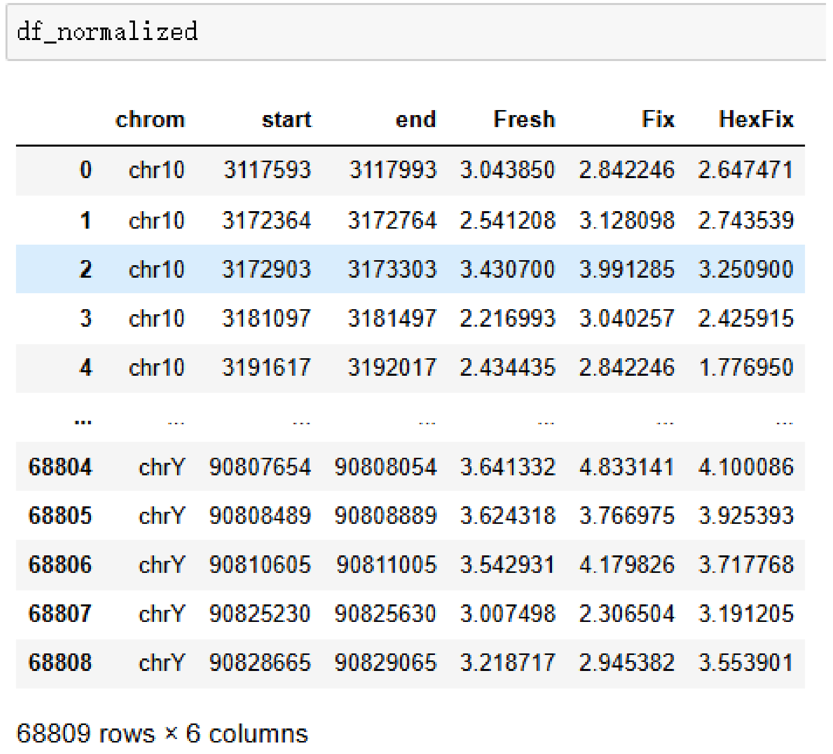


In the field of biological information, quantile normalization is commonly used(2). In this project, Median normalization (MED), or the 50-quantile normalization is used. This method lines up the median of each column to make the data be on the same “scale”.





After Normalization

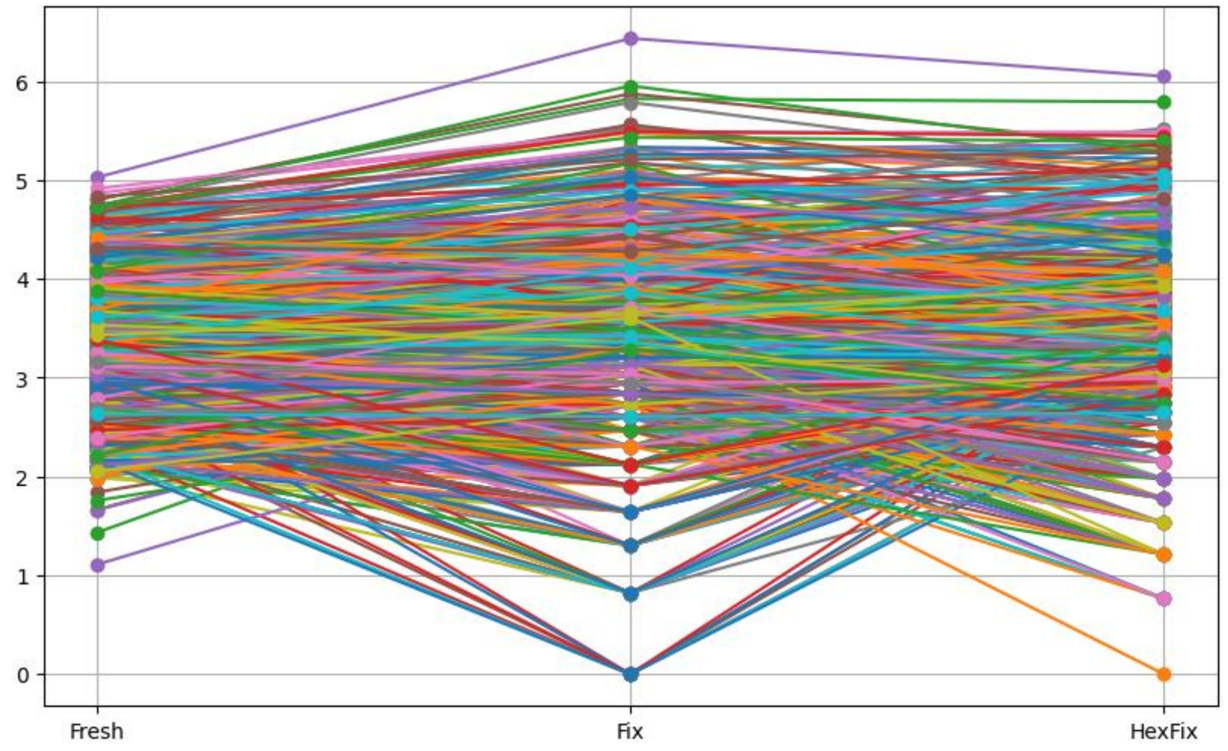


```
df_normalized
```

	chrom	start	end	Fresh	Fix	HexFix
0	chr10	3117593	3117993	3.043850	2.842246	2.647471
1	chr10	3172364	3172764	2.541208	3.128098	2.743539
2	chr10	3172903	3173303	3.430700	3.991285	3.250900
3	chr10	3181097	3181497	2.216993	3.040257	2.425915
4	chr10	3191617	3192017	2.434435	2.842246	1.776950
...	...	...	...	...	...	...
68804	chrY	90807654	90808054	3.641332	4.833141	4.100086
68805	chrY	90808489	90808889	3.624318	3.766975	3.925393
68806	chrY	90810605	90811005	3.542931	4.179826	3.717768
68807	chrY	90825230	90825630	3.007498	2.306504	3.191205
68808	chrY	90828665	90829065	3.218717	2.945382	3.553901

68809 rows × 6 columns

Line plot for the first 1000 rows



Data Transformation

Now we have 3 normalized columns. Before we feed the data to a clustering algorithm to generate the labels, it is better to apply certain transformations that better capture the feature we care about, and hopefully could improve the clustering result.

Some possible transformation methods are:

- Z-score transformation (does not do a significantly better job to capture wanted features, and might have problems)
- Difference transformation (simple and effective)
- HSV transformation (very good at capturing the “shape” pattern and cool)

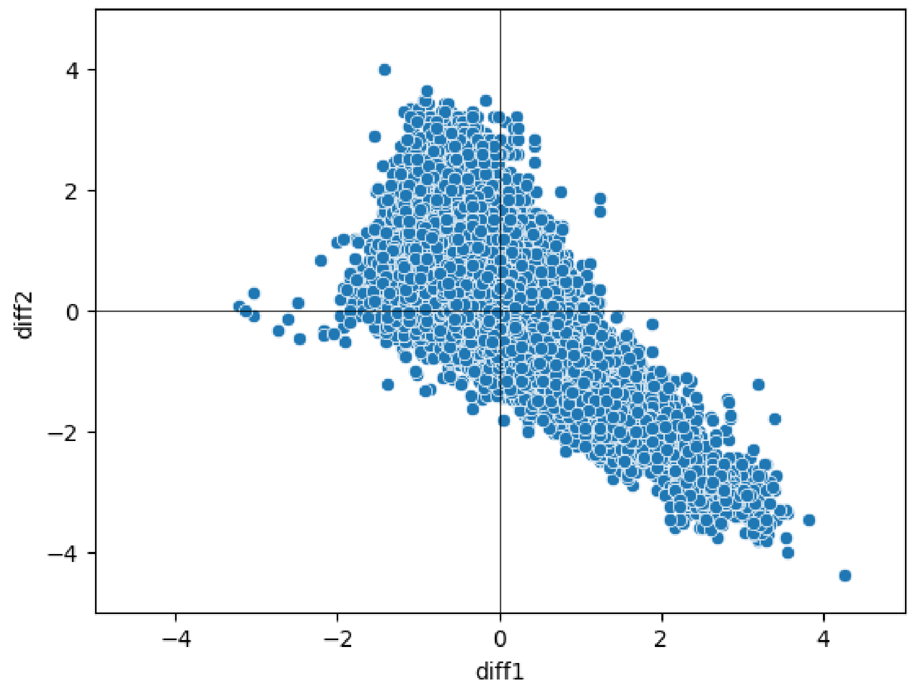
Difference transformation

Transform (Fresh, Fix, HexFix) to (Fresh - Fix, Fix - HexFix)

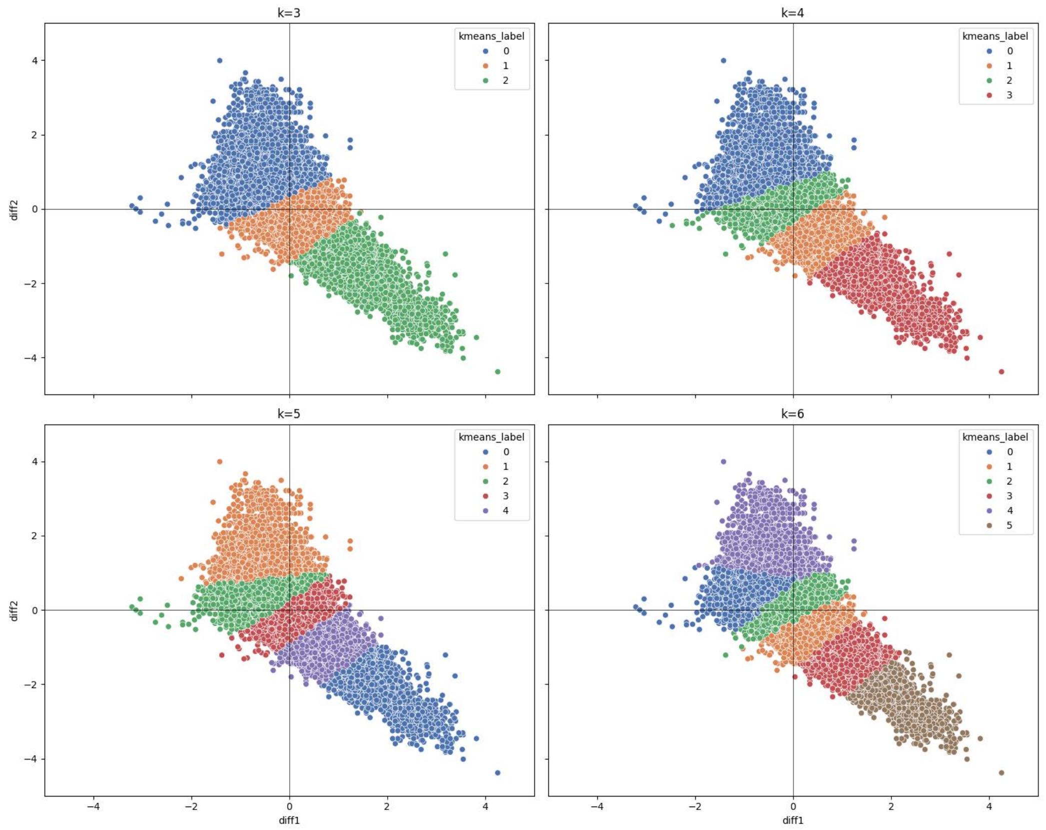
- Advantage: focuses on the difference feature we care about, dimension reduction, visualization possible
- Disadvantage: loss of information

Fresh	Fix	HexFix
3.043850	2.842246	2.647471
2.541208	3.128098	2.743539
3.430700	3.991285	3.250900
2.216993	3.040257	2.425915
2.434435	2.842246	1.776950
...	...	...
3.641332	4.833141	4.100086
3.624318	3.766975	3.925393
3.542931	4.179826	3.717768
3.007498	2.306504	3.191205
3.218717	2.945382	3.553901

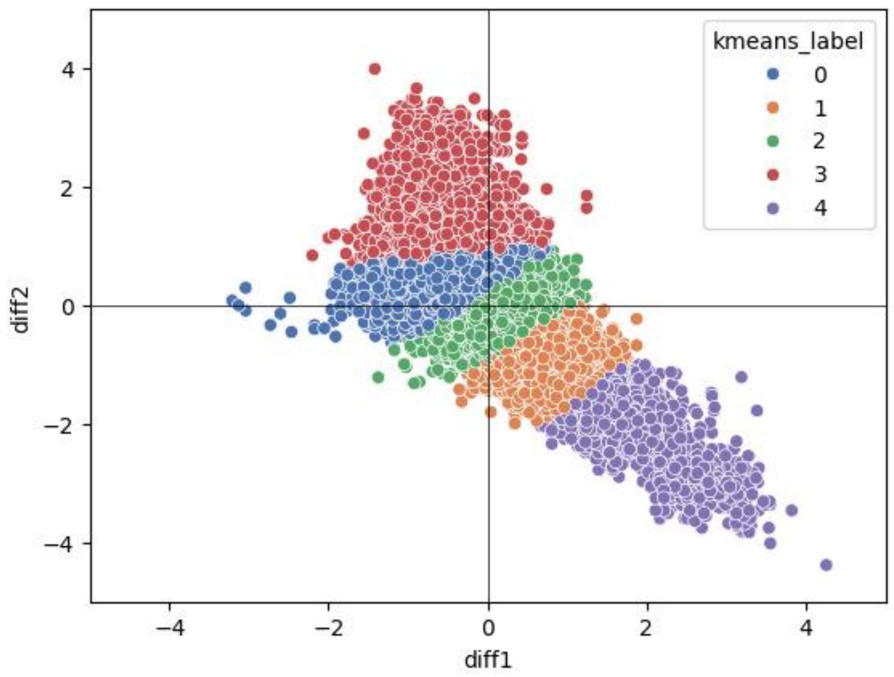
diff1	diff2
0.201604	0.194775
-0.586890	0.384559
-0.560585	0.740385
-0.823264	0.614342
-0.407812	1.065296
...	...
-1.191810	0.733056
-0.142657	-0.158418
-0.636895	0.462058
0.700994	-0.884701
0.273335	-0.608519



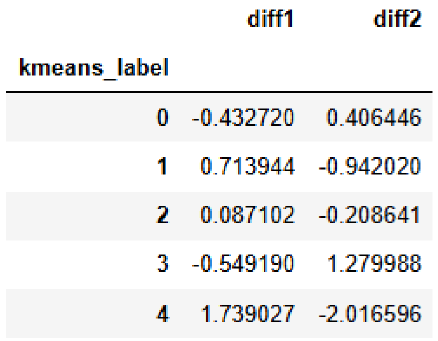
Kmeans



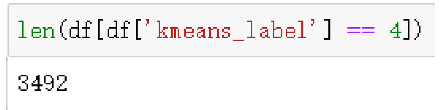
Kmeans result for k=5



Mean of each label



kmeans_label	diff1	diff2
0	-0.432720	0.406446
1	0.713944	-0.942020
2	0.087102	-0.208641
3	-0.549190	1.279988
4	1.739027	-2.016596

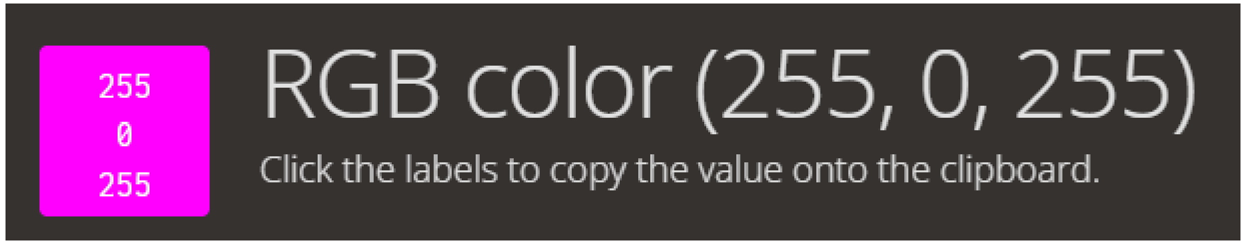


```
len(df[df['kmeans_label'] == 4])
```

```
3492
```

HSV transformation

Say we have a tuple (R, G, B) representing a color. Imagine you have a V-shape pattern for the RGB values (high R and B value, low G value), what color could it possibly look like?

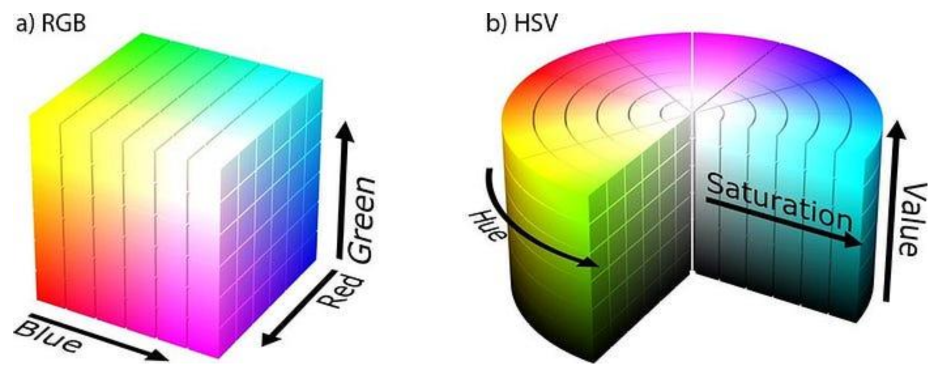

255
0
255

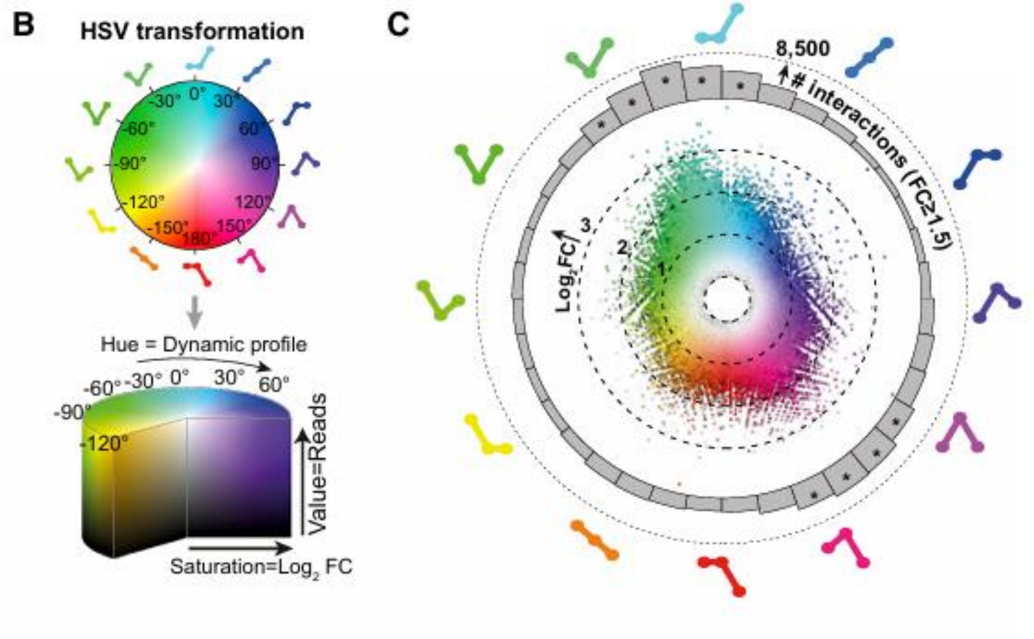
RGB color (255, 0, 255)

Click the labels to copy the value onto the clipboard.

Could it be that the V-shape pattern we are looking for is simply within a specific color range when converted into a RGB color?

Indeed this is the case! If we treat the (Fresh, Fix, HexFix) tuple as RGB values of a color, all V-shape tuples should represent a color within a certain color range, in other words, a color which hue is in a certain range (when transformed into a HSV color).





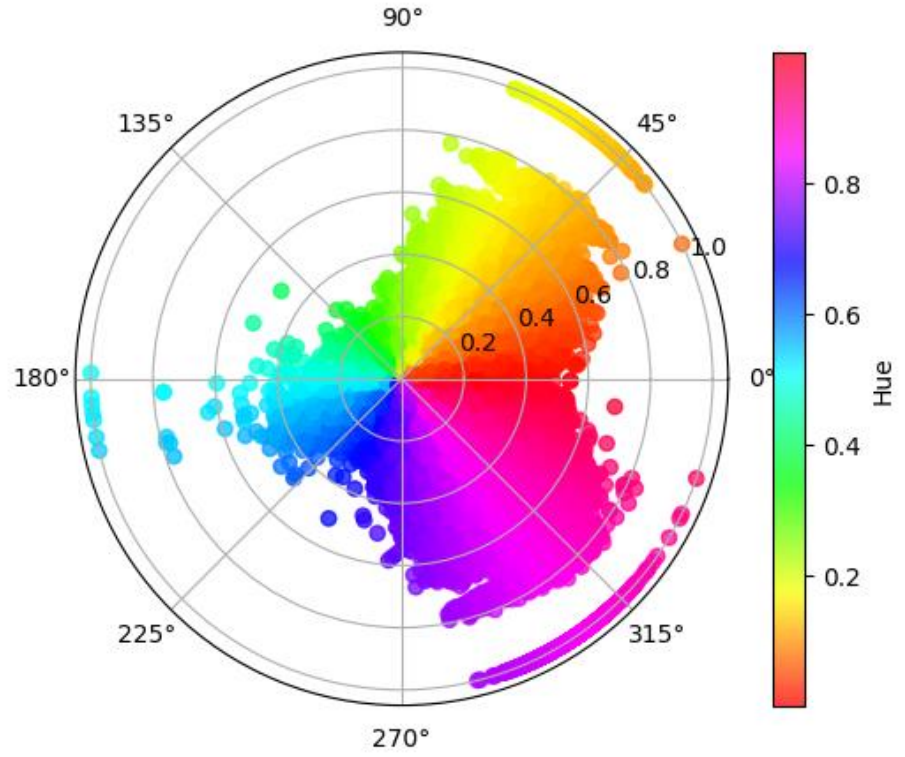
Siersbæk et al., 2017. $^{(3)}$

Fresh	Fix	HexFix
0.550832	0.387107	0.392360
0.459871	0.426039	0.406597
0.620838	0.543603	0.481789
0.401199	0.414075	0.359525
0.440549	0.387107	0.263347
...	...	...
0.658955	0.658261	0.607640
0.655877	0.513052	0.581750
0.641148	0.569282	0.550980
0.544253	0.314140	0.472942
0.582477	0.401153	0.526694

The columns are normalized to be between [0, 1] for convenience of HSV transformation

H	S	V
0.994652	0.297233	0.550832
0.060823	0.115845	0.459871
0.074091	0.223970	0.620838
0.206006	0.131740	0.414075
0.116402	0.402229	0.440549
...	...	...
0.164412	0.077874	0.658955
0.919834	0.217761	0.655877
0.033829	0.140636	0.641148
0.884983	0.422806	0.544253
0.884607	0.311297	0.582477

HSV values are also between [0, 1]

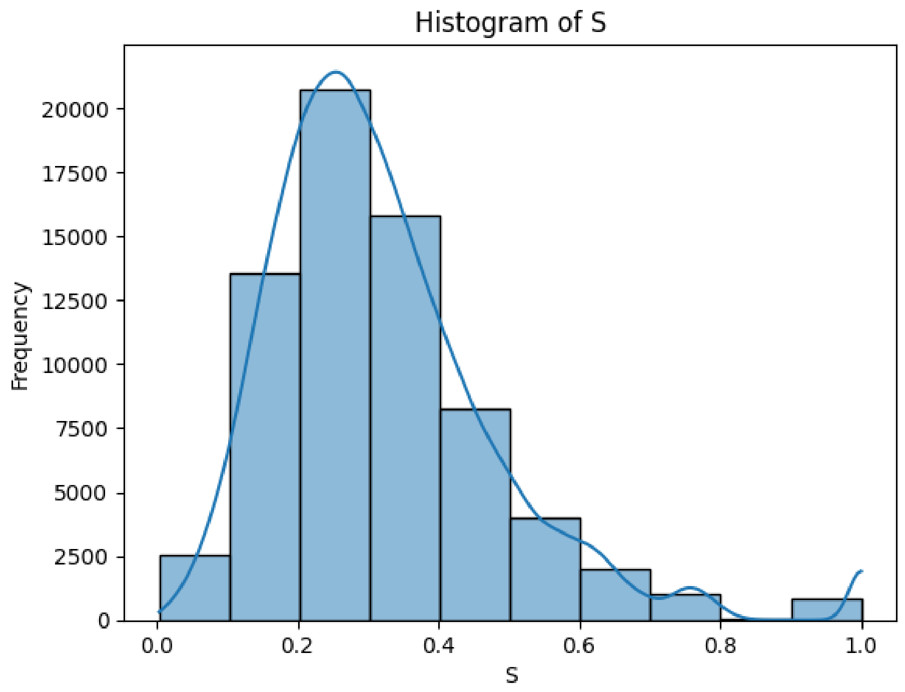


Interpretation of HSV transformation

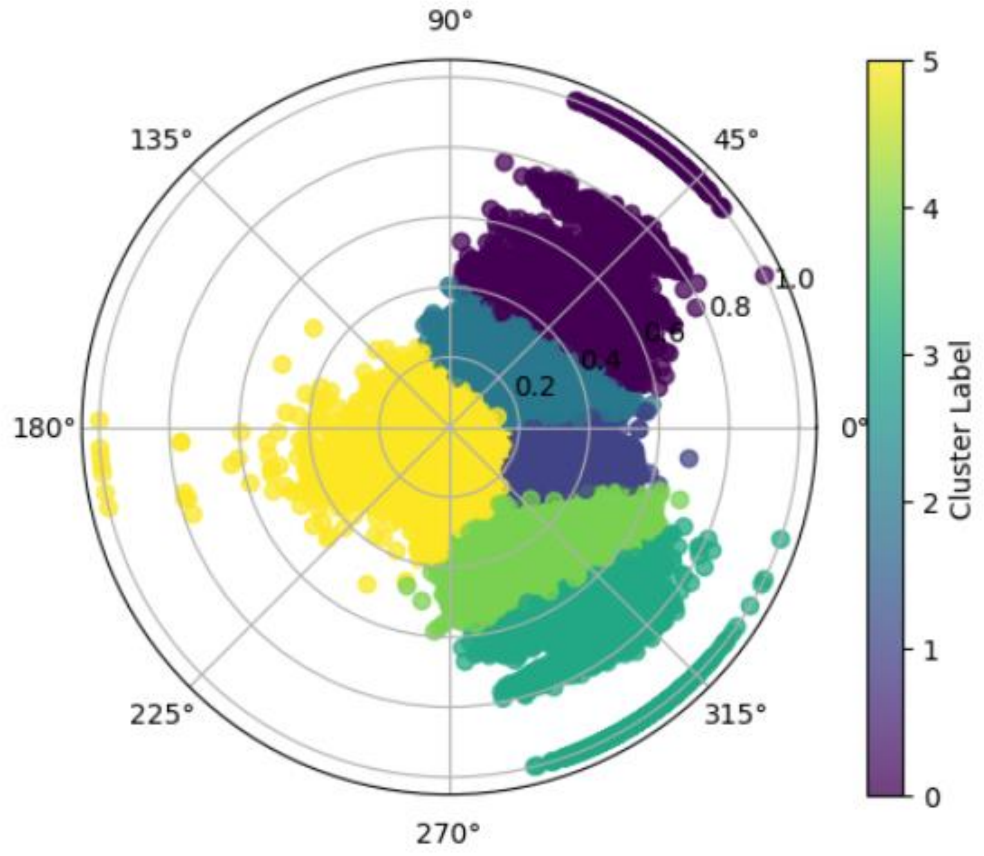
For a (R, G, B) to (H, S, V) transformation:

- H is the "shape" of the R-G-B angle
- S is (max – min) / max, where max, min are the maximum, minimum value in (R, G, B)
- V is the max value in (R, G, B)

Except for certain H range (the V-shape), we also look for certain S range (how deep the "V" is).



Kmeans



Mean of each label

hsv_label	Fresh	Fix	HexFix
0	0.496530	0.421150	0.232232
1	0.637153	0.454633	0.519214
2	0.579010	0.486980	0.427054
3	0.497304	0.141714	0.456892
4	0.551271	0.321013	0.495641
5	0.580395	0.513293	0.552799

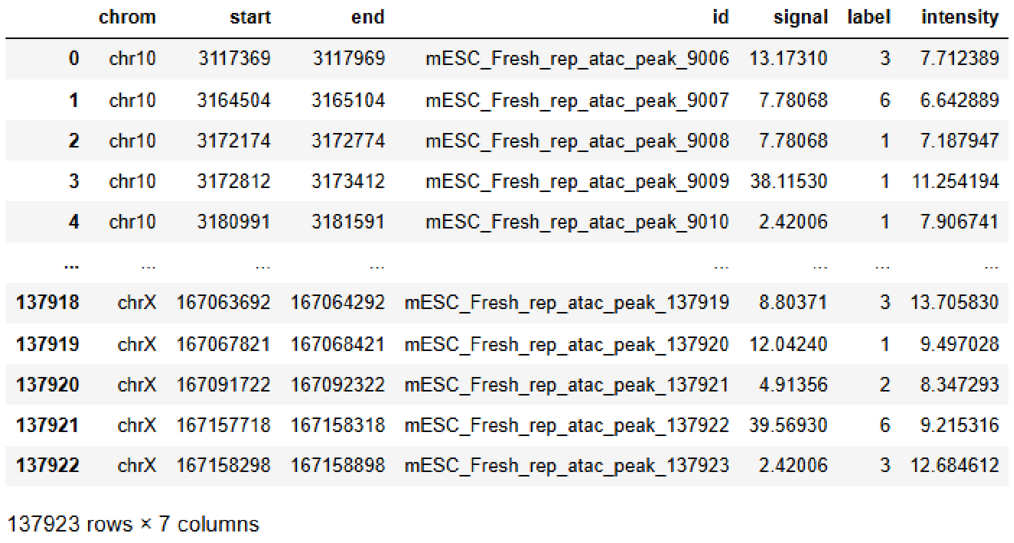
Count of each label

```
hsv_label
1     18744
2     17179
5     12801
4     12256
0      4508
3      3321
```

Data cleaning

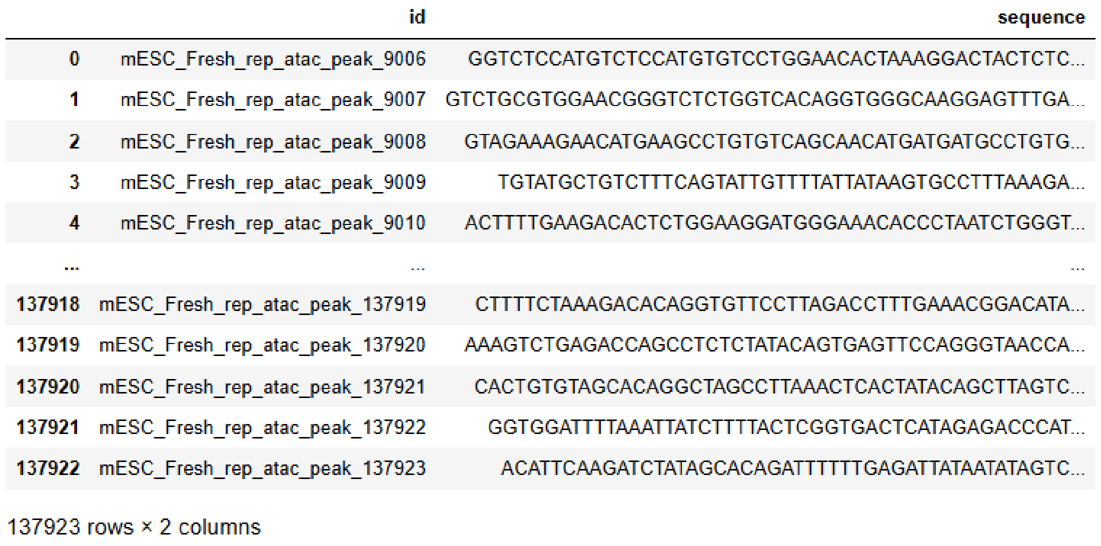
The second subset comes in a very strange format (at least strange to me): .bed and .fa file.

- .bed file can be read by pandas using the read_csv function and use '\t' as delimiter.
- .fa file is generated by a software called FASTA, which is wildly used in bioinformatics to store nucleotide or protein sequences. In python, it has to be read using special package functions (SeqIO function from Bio package).
- This is what the dataset looks like, it is thus easy to see that the two tables can join on a common key, id:



	chrom	start	end	id	signal	label	intensity
0	chr10	3117369	3117969	mESC_Fresh_rep_atac_peak_9006	13.17310	3	7.712389
1	chr10	3164504	3165104	mESC_Fresh_rep_atac_peak_9007	7.78068	6	6.642889
2	chr10	3172174	3172774	mESC_Fresh_rep_atac_peak_9008	7.78068	1	7.187947
3	chr10	3172812	3173412	mESC_Fresh_rep_atac_peak_9009	38.11530	1	11.254194
4	chr10	3180991	3181591	mESC_Fresh_rep_atac_peak_9010	2.42006	1	7.906741
...	...	...	...	...	...	...	...
137918	chrX	167063692	167064292	mESC_Fresh_rep_atac_peak_137919	8.80371	3	13.705830
137919	chrX	167067821	167068421	mESC_Fresh_rep_atac_peak_137920	12.04240	1	9.497028
137920	chrX	167091722	167092322	mESC_Fresh_rep_atac_peak_137921	4.91356	2	8.347293
137921	chrX	167157718	167158318	mESC_Fresh_rep_atac_peak_137922	39.56930	6	9.215316
137922	chrX	167158298	167158898	mESC_Fresh_rep_atac_peak_137923	2.42006	3	12.684612

137923 rows × 7 columns

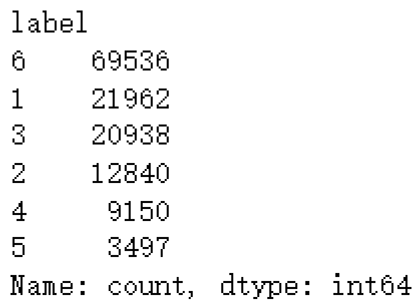


	id	sequence
0	mESC_Fresh_rep_atac_peak_9006	GGTCTCCATGTCTCCATGTGTCCTGGAACACTAAAGGACTACTCTC...
1	mESC_Fresh_rep_atac_peak_9007	GTCTGCGTGGAACGGGTCTCTGGTCACAGGTGGGCAAGGAGTTTGA...
2	mESC_Fresh_rep_atac_peak_9008	GTAGAAAGAACATGAAGCCTGTGTCAGCAACATGATGATGCCTGTG...
3	mESC_Fresh_rep_atac_peak_9009	TGTATGCTGTCTTTCAGTATTGTTTTATTATAAGTGCCTTTAAAGA...
4	mESC_Fresh_rep_atac_peak_9010	ACTTTTGAAGACACTCTGGAAGGATGGGAAACACCCTAATCTGGGT...
...	...	...
137918	mESC_Fresh_rep_atac_peak_137919	CTTTTCTAAAGACACAGGTGTTCCTTAGACCTTTGAAACGGACATA...
137919	mESC_Fresh_rep_atac_peak_137920	AAAGTCTGAGACCAGCCTCTCTATACAGTGAGTTCCAGGGTAACCA...
137920	mESC_Fresh_rep_atac_peak_137921	CACTGTGTAGCACAGGCTAGCCTTAAACTCACTATACAGCTTAGTC...
137921	mESC_Fresh_rep_atac_peak_137922	GGTGGATTTTAAATTATCTTTTACTCGGTGACTCATAGAGACCCAT...
137922	mESC_Fresh_rep_atac_peak_137923	ACATTCAAGATCTATAGCACAGATTTTTTGAGATTATAATATAGTC...

137923 rows × 2 columns

There are some problems to be solved before this data could be used to train a model. First, the labels of the peaks are from 1 to 6, while labels 1 to 5 represent class 0 to 4 in the Kmeans result in former step, while label 6 represents “unlabeled”. Thus, we have to delete all rows with label 6 (this is nearly half of the data), and reduce all label by 1 to get the original label we generated from the clustering algorithm.

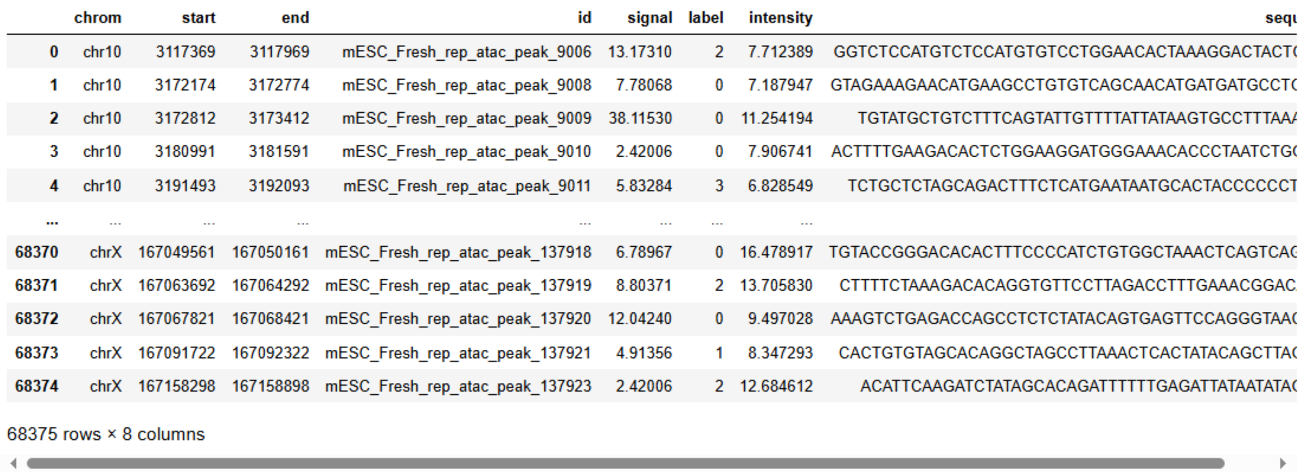
I also swap label 0 with label 4, so that label 0 now represents the V-shape we are looking for. This saves time for me to remember which label is the label we target at.



```
label
6    69536
1    21962
3    20938
2    12840
4     9150
5     3497
Name: count, dtype: int64
```

Another problem is that, besides A, T, C, and G, there is also a N nucleotide in the sequence column, which represents “unknown” or “any” nucleotide. We do not want this noise in our model, so I want to delete all rows whose sequence contains the N nucleotide.

This is what the data looks like after data cleaning:



	chrom	start	end	id	signal	label	intensity	sequ
0	chr10	3117369	3117969	mESC_Fresh_rep_atac_peak_9006	13.17310	2	7.712389	GGTCTCCATGTCTCCATGTGTCCTGGAACACTAAAGGACTACT
1	chr10	3172174	3172774	mESC_Fresh_rep_atac_peak_9008	7.78068	0	7.187947	GTAGAAAGAACATGAAGCCTGTGTCAGCAACATGATGATGCCT
2	chr10	3172812	3173412	mESC_Fresh_rep_atac_peak_9009	38.11530	0	11.254194	TGTATGCTGTCTTTCAGTATTGTTTTATTATAAGTGCCTTTAA
3	chr10	3180991	3181591	mESC_Fresh_rep_atac_peak_9010	2.42006	0	7.906741	ACTTTTGAAGACACTCTGGAAGGATGGGAAACACCCTAATCTG
4	chr10	3191493	3192093	mESC_Fresh_rep_atac_peak_9011	5.83284	3	6.828549	TCTGCTCTAGCAGACTTTCTCATGAATAATGCACTACCCCCCT
...	...	...	...	...	...	...	...	
68370	chrX	167049561	167050161	mESC_Fresh_rep_atac_peak_137918	6.78967	0	16.478917	TGTACCGGGACACACTTTCCCCATCTGTGGCTAAACTCAGTCA
68371	chrX	167063692	167064292	mESC_Fresh_rep_atac_peak_137919	8.80371	2	13.705830	CTTTTCTAAAGACACAGGTGTTCCTTAGACCTTTGAAACGGAC
68372	chrX	167067821	167068421	mESC_Fresh_rep_atac_peak_137920	12.04240	0	9.497028	AAAGTCTGAGACCAGCCTCTCTATACAGTGAGTTCCAGGGTAA
68373	chrX	167091722	167092322	mESC_Fresh_rep_atac_peak_137921	4.91356	1	8.347293	CACTGTGTAGCACAGGCTAGCCTTAAACTCACTATACAGCTTA
68374	chrX	167158298	167158898	mESC_Fresh_rep_atac_peak_137923	2.42006	2	12.684612	ACATTCAAGATCTATAGCACAGATTTTTTGAGATTATAATATA

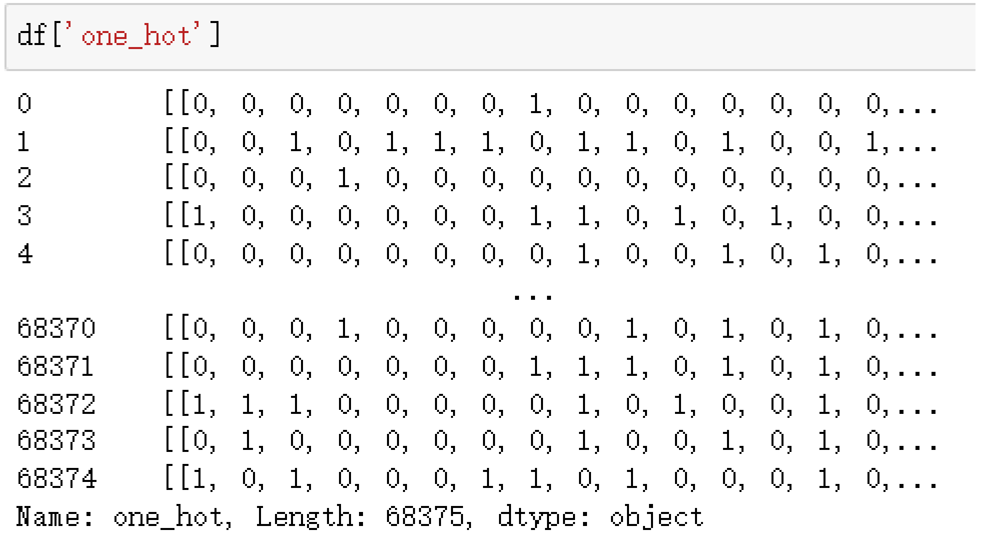
68375 rows × 8 columns

While we got rid of nearly half the data, we still have about the same amount of rows in remain comparing to the first dataset, so it’s not too bad.

One-hot encoding

A common approach to extract patterns from a sequence in bioinformatics is to one-hot encode the sequence, so that a sequence of length n becomes a matrix of 4*n. This matrix is then fed into a CNN to extract the spatial information for further analysis. The methodology used in this project has referred to a paper by Han Yuan and David R. Kelley (2022)(4).

This is how the one-hot encoding looks like:



```
df['one_hot']
```

```
0        [[0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0,...
1        [[0, 0, 1, 0, 1, 1, 1, 0, 1, 1, 0, 1, 0, 0, 1,...
2        [[0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,...
3        [[1, 0, 0, 0, 0, 0, 0, 1, 1, 0, 1, 0, 1, 0, 0,...
4        [[0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 1, 0, 1, 0,...
                                 ...
68370    [[0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 0, 1, 0, 1, 0,...
68371    [[0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 0, 1, 0, 1, 0,...
68372    [[1, 1, 1, 0, 0, 0, 0, 0, 1, 0, 1, 0, 0, 1, 0,...
68373    [[0, 1, 0, 0, 0, 0, 0, 0, 1, 0, 0, 1, 0, 1, 0,...
68374    [[1, 0, 1, 0, 0, 0, 1, 1, 0, 1, 0, 0, 0, 1, 0,...
Name: one_hot, Length: 68375, dtype: object
```

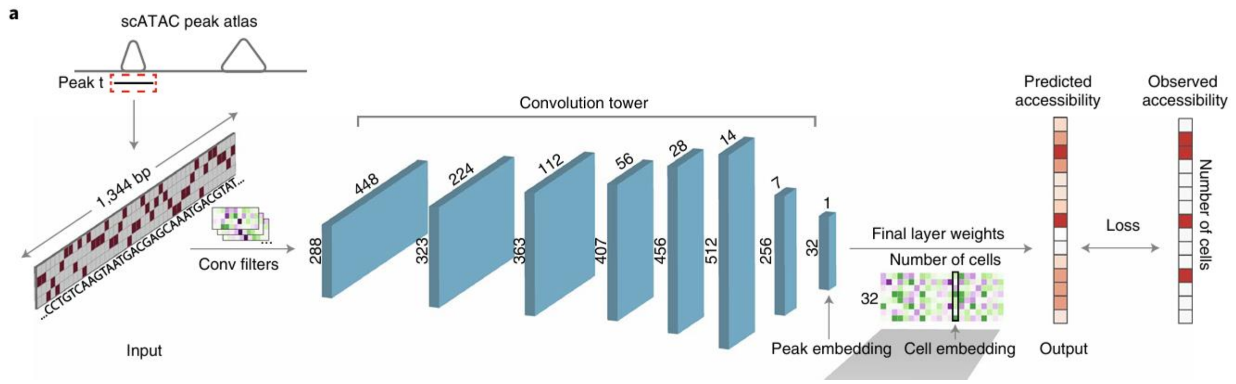
CNN architecture

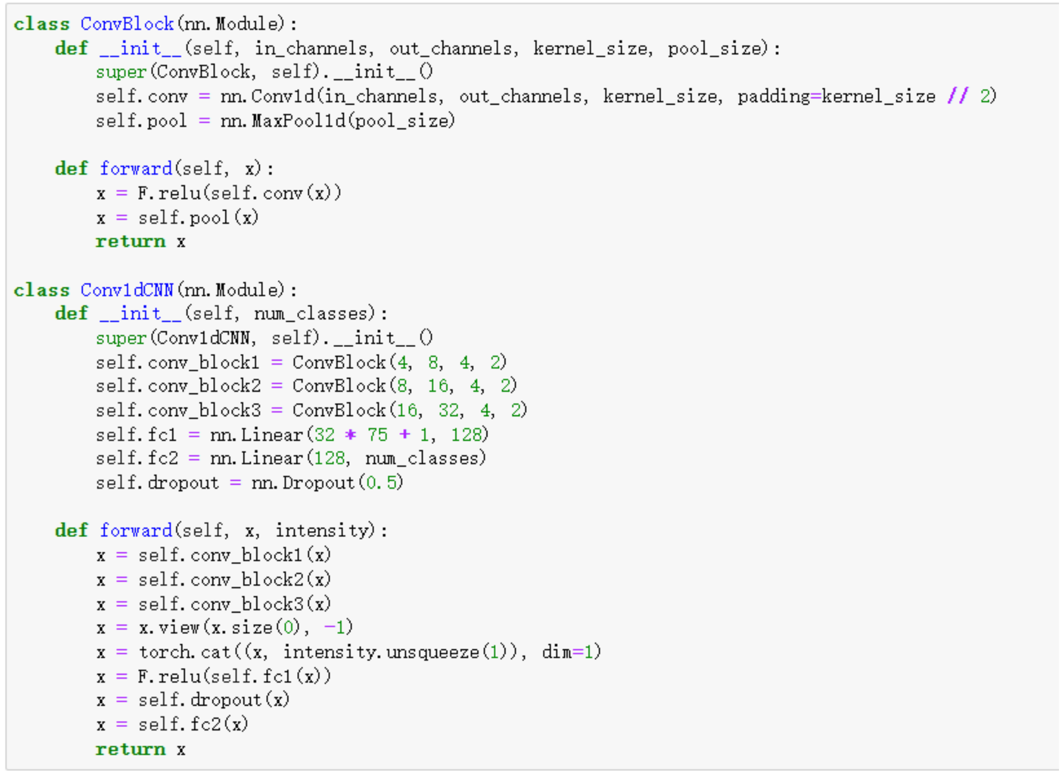
Because of the property of one-hot encoding, namely that each column can only have one "1" entry, the row-wise (or between-row) relationship is naturally of less significance than column-wise (or within-row) relationship. This property has led to a certain kind of CNN architecture we use in this project.

Instead of the Conv2d layer, which has a 2d kernel and is usually used on spatial data, here we use Conv1d layer, which has a 1d kernel and is usually used on sequential data. In this specific example, we treat each row as a individual sequence and apply Conv1d layer to it to get a new sequence for each channel. This approach has a corresponding bioinformatics significance: for example, the first row could be interpreted as the distribution of 'A' nucleotide.

CNN architecture

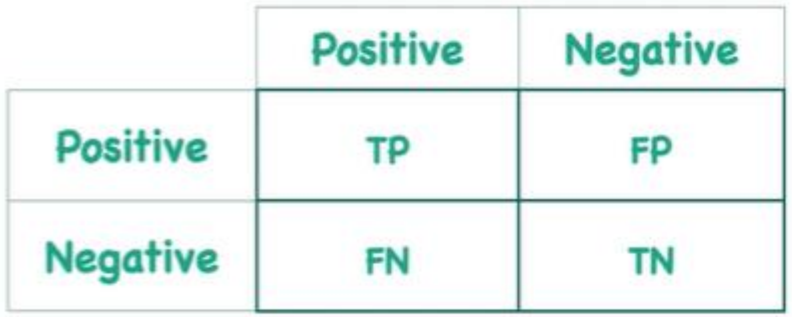
The CNN architecture design also have referred to the paper by Han Yuan and David R. Kelley (2022)[4].



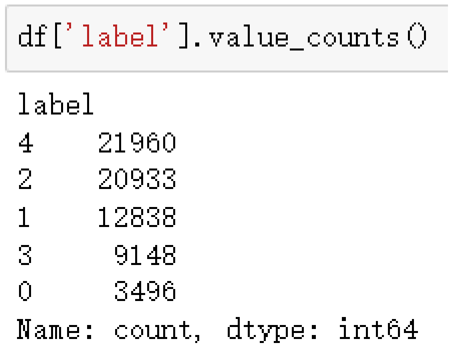

```python
class ConvBlock(nn.Module):
    def __init__(self, in_channels, out_channels, kernel_size, pool_size):
        super(ConvBlock, self).__init__()
        self.conv = nn.Conv1d(in_channels, out_channels, kernel_size, padding=kernel_size // 2)
        self.pool = nn.MaxPool1d(pool_size)

    def forward(self, x):
        x = F.relu(self.conv(x))
        x = self.pool(x)
        return x

class Conv1dCNN(nn.Module):
    def __init__(self, num_classes):
        super(Conv1dCNN, self).__init__()
        self.conv_block1 = ConvBlock(4, 8, 4, 2)
        self.conv_block2 = ConvBlock(8, 16, 4, 2)
        self.conv_block3 = ConvBlock(16, 32, 4, 2)
        self.fc1 = nn.Linear(32 * 75 + 1, 128)
        self.fc2 = nn.Linear(128, num_classes)
        self.dropout = nn.Dropout(0.5)

    def forward(self, x, intensity):
        x = self.conv_block1(x)
        x = self.conv_block2(x)
        x = self.conv_block3(x)
        x = x.view(x.size(0), -1)
        x = torch.cat((x, intensity.unsqueeze(1)), dim=1)
        x = F.relu(self.fc1(x))
        x = self.dropout(x)
        x = self.fc2(x)
        return x
```

Training and evaluation

- Data is randomly sampled from the original dataset with a 80%/20% train/test split ratio
- Default sample size 20000 (16000 training data and 4000 validation data)
- Use cross entropy loss
- Learning rate set to be 0.001, with 1e-4 weight decay
- Train 20 epochs, record the training and validation loss for each epoch, plot the loss when finished
- Evaluate on both the overall accuracy and the accuracy on our target label (label 0 in this case) to prevent the model **from biased of unbalanced label amount**



	Positive	Negative
Positive	TP	FP
Negative	FN	TN

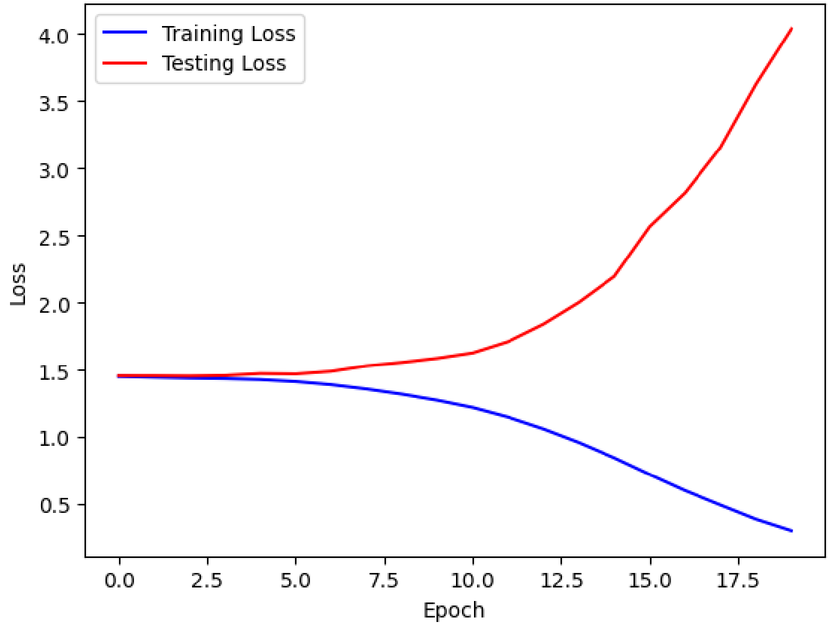


```
df['label'].value_counts()
```

```
label
4    21960
2    20933
1    12838
3     9148
0     3496
Name: count, dtype: int64
```

Result

Long story short, the CNN model does not work out well. The validation loss almost always goes up immediately after the training loss starts to go down (as shown below). This is a strong sign of overfitting in ML, where the model simply "remember" the data instead of "learn" the underlying pattern, which makes the model impossible to generalize to unseen data.



```
Overall Accuracy: 0.2542
Correct Label: 1017.0000
Total Samples: 4000.0000
Accuracy on label 0: 0.0830
Correct 0 Label: 19.0000
Total 0 Sample: 229.0000
```

Discussion

Several possible reasons might lead to this result:

- There’s a problem with the model
  - The model is too complicated for the data
  - The model architecture does not fit the data
- There’s a problem with the data
  - The data has too little pattern/too much noise
  - The labels have poor quality

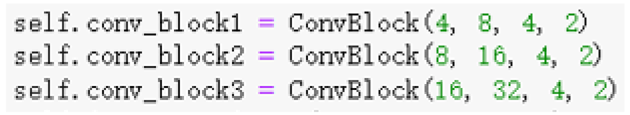
Tuning model complexity

There is a relative relationship between the amount of training data and the complexity of the model for the model to be able to completely remember the data. To ameliorate the situation, we could work from both sides:

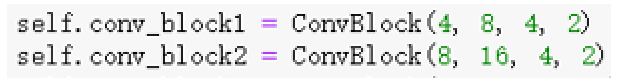
- To feed more data
- To simplify the model
  - Reduce the number of parameters in the convolutional layers
  - Add regularization (already applied in the original model)
  - Add dropout layer

If the loss curve shows significantly different pattern with more data/simpler architecture, then model complexity could very possibly be the true cause to this overfitting outcome.

Simplifying Convolutional layer



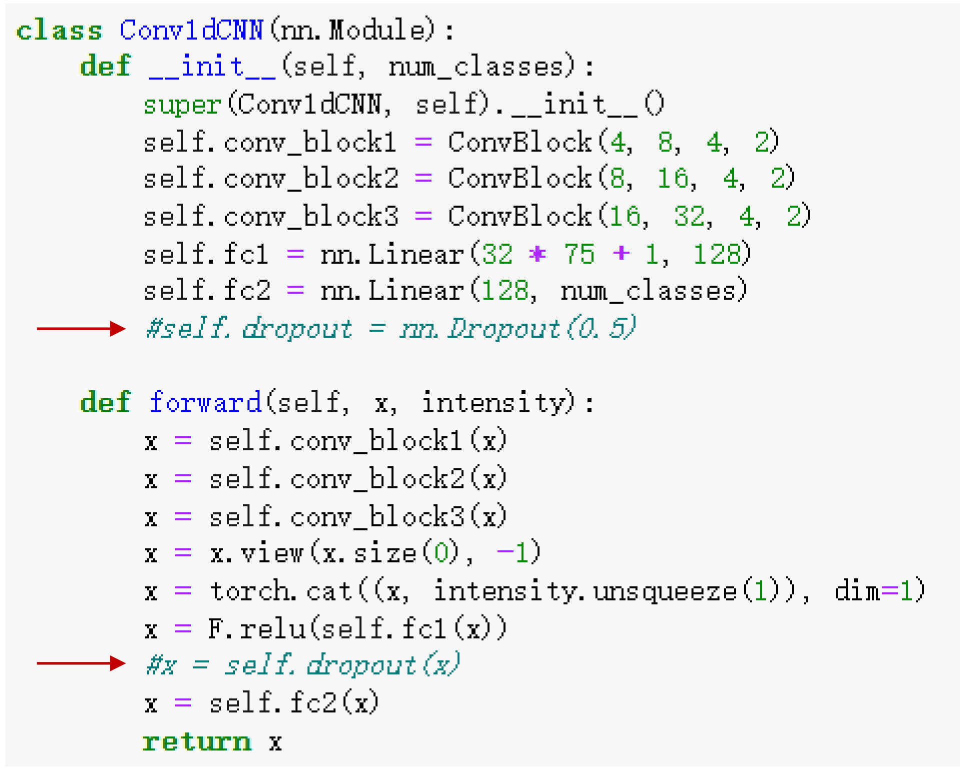
```
self.conv_block1 = ConvBlock(4, 8, 4, 2)
self.conv_block2 = ConvBlock(8, 16, 4, 2)
self.conv_block3 = ConvBlock(16, 32, 4, 2)
```



```
self.conv_block1 = ConvBlock(4, 8, 4, 2)
self.conv_block2 = ConvBlock(8, 16, 4, 2)
```

Although the deletion of “self.conv_block3” does not change the number of input parameters to the first fully connected layer (from 32*75+1 to 16*150+1), it directly remove all parameters within this layer ((16*2+1)*32 parameters, more than the first 2 convolutional layers added up). By eliminating more than half the parameters in the convolutional layers, this approach significantly reduce the complexity of the model.

Adding Dropout Layer

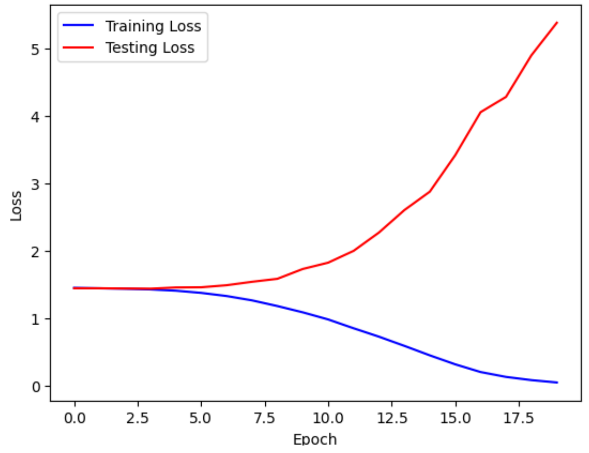


```
class Conv1dCNN(nn.Module):
    def __init__(self, num_classes):
        super(Conv1dCNN, self).__init__()
        self.conv_block1 = ConvBlock(4, 8, 4, 2)
        self.conv_block2 = ConvBlock(8, 16, 4, 2)
        self.conv_block3 = ConvBlock(16, 32, 4, 2)
        self.fc1 = nn.Linear(32 * 75 + 1, 128)
        self.fc2 = nn.Linear(128, num_classes)
        #self.dropout = nn.Dropout(0.5)

    def forward(self, x, intensity):
        x = self.conv_block1(x)
        x = self.conv_block2(x)
        x = self.conv_block3(x)
        x = x.view(x.size(0), -1)
        x = torch.cat((x, intensity.unsqueeze(1)), dim=1)
        x = F.relu(self.fc1(x))
        #x = self.dropout(x)
        x = self.fc2(x)
        return x
```

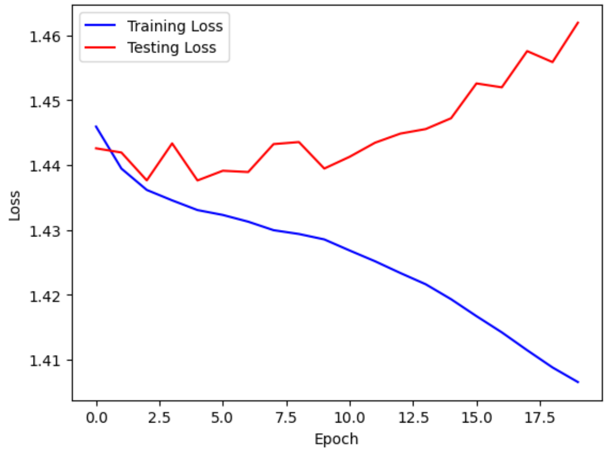
The dropout layer is conventionally added in between the two fully connected layers with a certain dropout rate. Here, I'm using a 0.5 dropout rate, which means 50% of the output parameters of "self.fc1" would be randomly set to be 0 during each training cycle. This significantly prevent overfitting.
Usually, in CNN, we use a smaller dropout rate (0.3 for example), because we want to preserve as much information as possible from the convolutional blocks.

Result

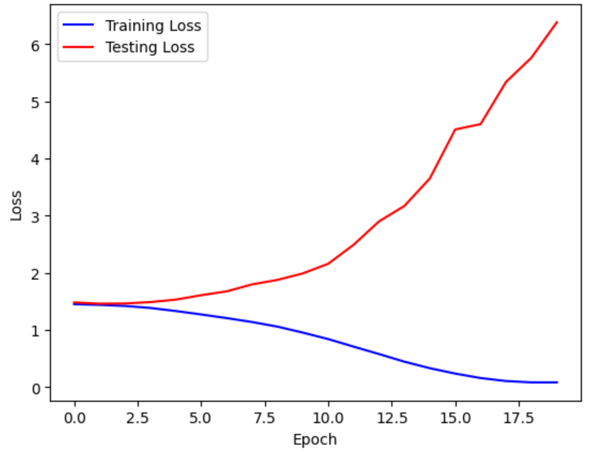
10k training data



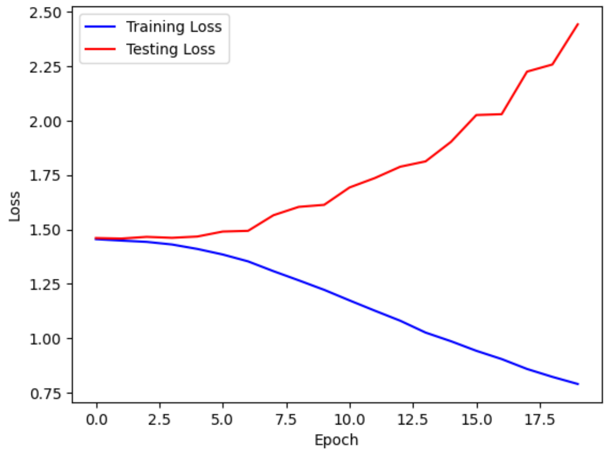
20k training data with 1 less convolutional layer



60k training data



20k training data with 0.5 dropout rate



Discussion

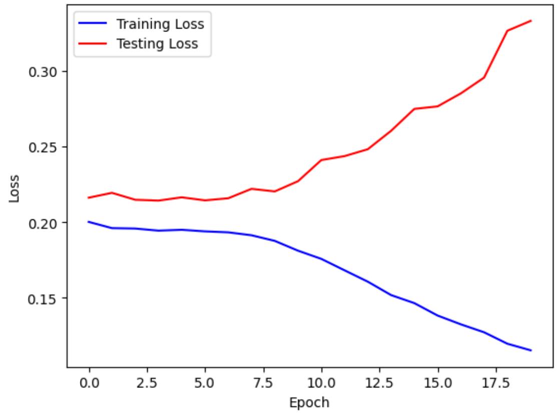
From the result we can see, no matter how we play with the hyperparameters or network architecture, the curve always looks roughly the same. The two right graphs looks very similar to the first few epochs of the left graphs, indicating that removing a convolutional layer or drop out 50% output from the first fulling connected layer only slows down the process of data being remembered. This indicates that the tuning the model architecture does not change much the “pattern” of the model remembering the data.

Given that CNN is a very common approach to learn DNA and protein sequence information in the field of bioinformatics, I conclude that model architecture is very unlikely to be the main reason for the overfitting problem.

Checking Label Validity

There is already a fair amount of introduced bias during the labeling process, yet I consider it the necessary approach for getting the labels we want. For example, normalization is necessary if we want to compare accessibilities under different experimental conditions, and we have to manually select k for the clustering algorithm, which is the same as manually set the boundary (this part of bias is greatly reduced with HSV transformation).

All that being said, it is still possible to improve the labels. The only pattern we care about is the V-shape pattern (aka. label 0), thus we can group all other labels to be simply label 1. By doing so, labeling noise is reduced, but at the same time, it raises another problem: there are only about 3.5k label 0's but 65k label 1's. This unbalanced label distribution might bias the model to predict label 1.



```
Overall Accuracy: 0.9245
Correct Label: 3698.0000
Total Samples: 4000.0000
Accuracy on label 0: 0.0218
Correct 0 Label: 5.0000
Total 0 Sample: 229.0000
```

If we want to train the model with the new label distribution, we will have to **punish false negative on label 0** more heavily than before.

Focal Loss

To solve the problem of imbalanced labels, we use Focal Loss instead of standard Cross-Entropy Loss to train our model. Focal Loss is given as (5):

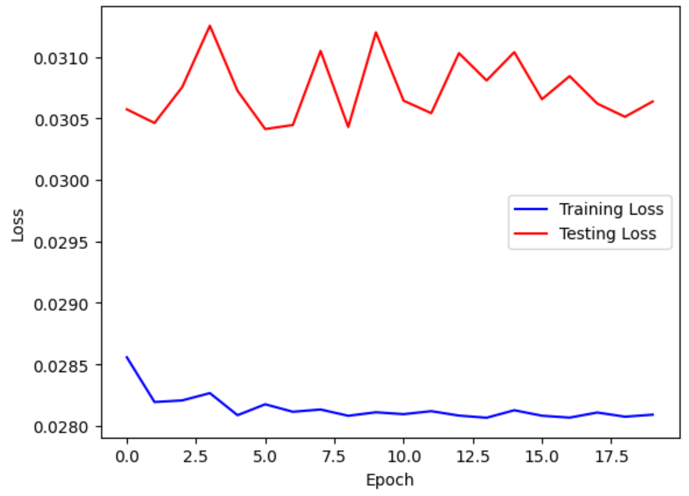
$$\mathrm{FL}(p_t) = -\alpha_t (1 - p_t)^\gamma \log(p_t).$$

By assigning different α values to different classes, Focal Loss could place unequal emphasis on the classes. On the other hand, γ controls for how much the model focuses on hard examples. In this project, we set γ conventionally to be 2, while tuning hyperparameter α, which is initially set to be [0.75, 0.25].

α could be set arbitrarily, the larger α_0/ α_1, the heavier we focus on correctly predict label 0.

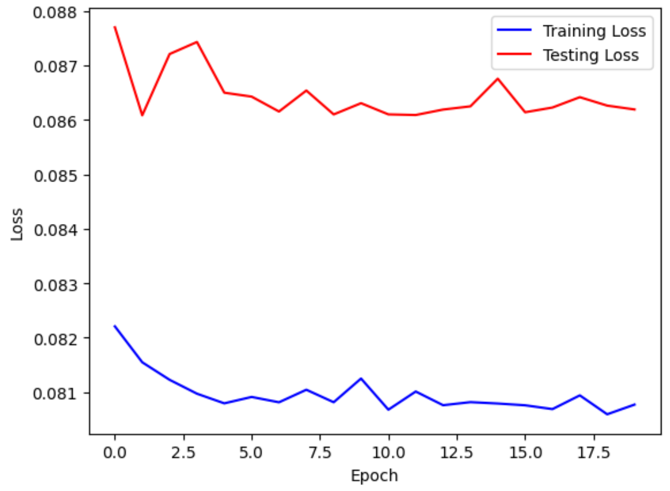
Result

$\alpha = [0.75, 0.25]$



```
Overall Accuracy: 0.9427
Correct Label: 3771.0000
Total Samples: 4000.0000
Accuracy on label 0: 0.0000
Correct 0 Label: 0.0000
Total 0 Sample: 229.0000
```

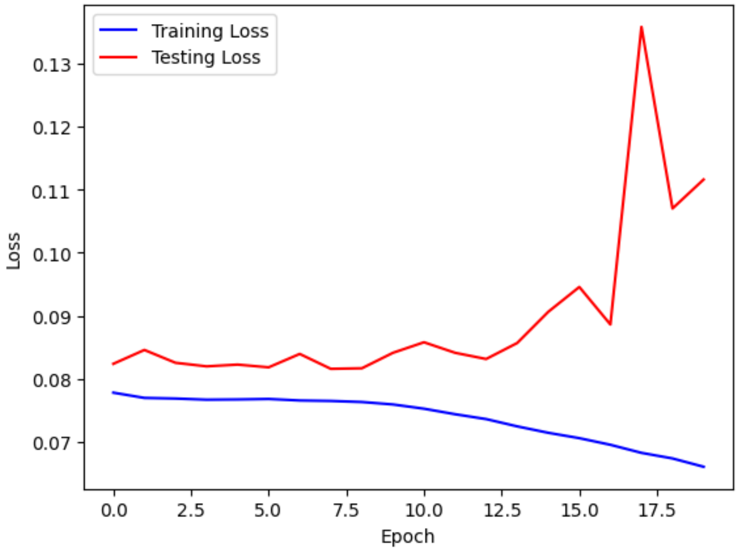
$\alpha = [5.0, 0.25]$



```
Overall Accuracy: 0.2575
Correct Label: 1030.0000
Total Samples: 4000.0000
Accuracy on label 0: 0.9432
Correct 0 Label: 216.0000
Total 0 Sample: 229.0000
```

When α_0/ α_1 is set too low or too high, the loss converges very quick, and the overall accuracy shows an extremely opposite pattern comparing to the accuracy on label 0. To eliminate the affect of unbalanced label, we want the two to be as close as possible.

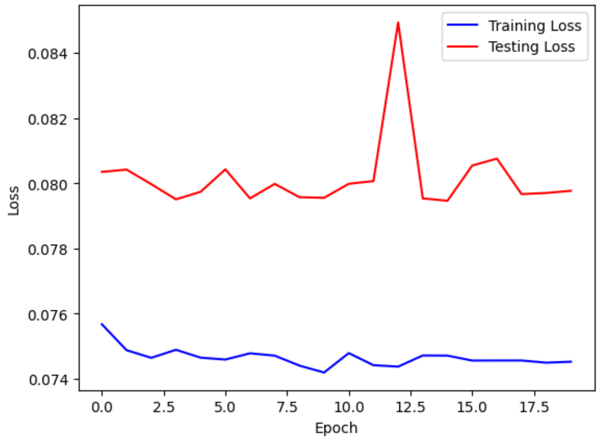
α = [4.5, 0.25]



```
Overall Accuracy: 0.5350
Correct Label: 2140.0000
Total Samples: 4000.0000
Accuracy on label 0: 0.5502
Correct 0 Label: 126.0000
Total 0 Sample: 229.0000
```

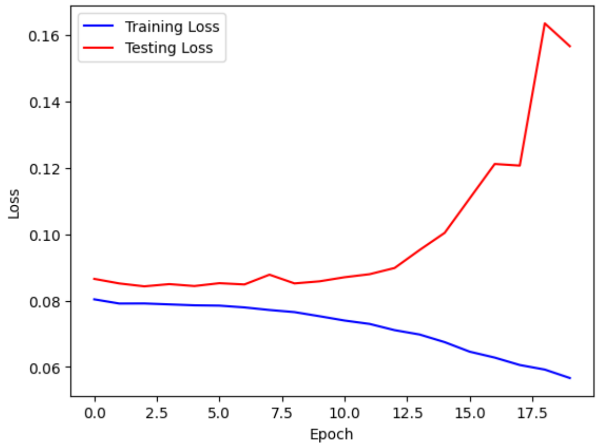
$\alpha_0/\ \alpha_1$-

$\alpha_0/\ \alpha_1$+



α = [4.25, 0.25]

```
Overall Accuracy: 0.4328
Correct Label: 1731.0000
Total Samples: 4000.0000
Accuracy on label 0: 0.7380
Correct 0 Label: 169.0000
Total 0 Sample: 229.0000
```



α = [4.75, 0.25]

```
Overall Accuracy: 0.6272
Correct Label: 2509.0000
Total Samples: 4000.0000
Accuracy on label 0: 0.4017
Correct 0 Label: 92.0000
Total 0 Sample: 229.0000
```

Discussion

From the result above we can see that when we set α to be [4.5, 0.25], the overall accuracy was very close to the accuracy on label 0, meaning that we managed to cancel the effect of imbalanced labels. Yet, both accuracies are slightly above 50%, which means that even without the effect of imbalanced labels, our model is merely better than a random classifier. Also, there is still obvious pattern of overfitting.

Using Focal Loss instead of Cross-Entropy Loss, I have controlled the random noise from the labeling step to the best I could. This has not contributed to improving overfitting, thus I conclude that label quality is not a major cause to the overfitting problem.

Future plan

Now that we have eliminated the following hypothesizes of the cause of overfitting:

- The model architecture is not a good fit
- The model is too complex
- The labels have poor quality

I will say that at this point, the most possible approach is to dig deeper into feature engineering of the original data. The same conclusion has been submitted to my research team, and my co-researchers are now working on capturing the complete distribution of intensity for each peak. This would result in a new channel with not only 0 and 1's, but real number entries of length of the peaks to feed into the network. I expect it to make up for the lack of pattern in our current data and to become the possible fix for the overfitting problem.

Addressing Peer Review Comments

The most common comment (tongue twister?) I got from peer reviews was that this project did not follow a clear claim-evidence-reason story line. This is because that the project is part of a larger, ongoing research project, and I was only half way through my works at the point of the first draft presentation, so that no particular claims could be made. In this presentation, you could clearly see that I was finally able to make claims about what I thought was the reason for the overfitting problem.

Other comments including ambiguous wording, lack of explanations on choice of methodology, and unclear interpretation of analytic results were also addressed in this presentation.

One comment I found hard to address was that there were too much text with too few graphs and plots. Although I used a presentational format, this project should in fact be written as a formal research article. There was not as much to show directly to the readers as a data analysis article, but much more to interpret and discuss. Thus, in this executive level presentation, I still did not include many visualizations, but wrote long interpretational paragraphs instead.

Links

- Project repository: https://github.com/N1colTeng/DATA-294P

Reference

(1) He et al., 2024, Cell. Dual-role transcription factors stabilize intermediate expression levels.

(2) Beibei Wang, Fengzhu Sun & Yihui Luan, 2024. Comparison of the effectiveness of different normalization methods for metagenomic cross-study phenotype prediction under heterogeneity.

(3) Siersbæk et al., 2017, Molecular Cell 66. DynamicRewiring of Promoter-Anchored Chromatin Loops during Adipocyte Differentiation.

(4) Han Yuan and David R. Kelley, 2022. scBasset: sequence-based modeling of single-cell ATAC-seq using convolutional neural networks.

(5) Tsung-Yi Lin et al., 2017. Focal Loss for Dense Object Detection.