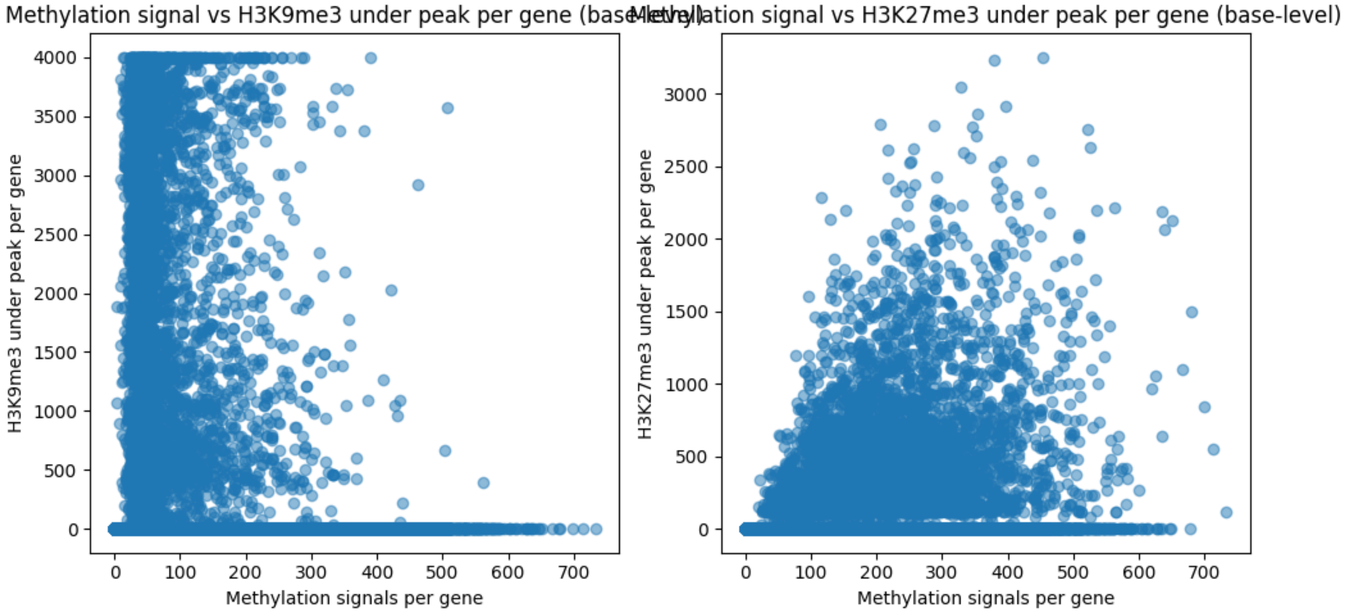
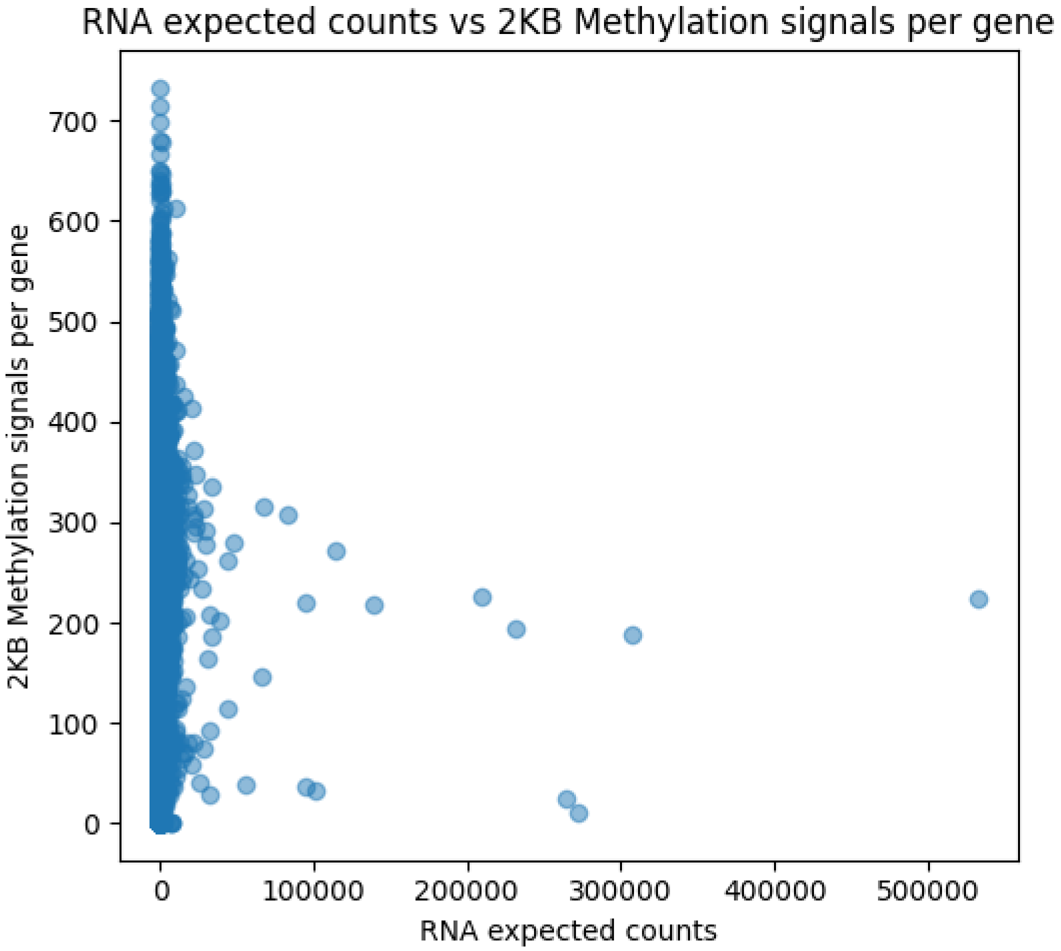
Results

Exploratory Analysis

- Plotting expected counts, DNAm and histone peaks against one another
- Adjusted gene annotations to +-2KB around TSS





Initial modelling

Utilising a 58780, 4000, 3 matrix created from the data

- 58780 genes
- 4000 nucleotides per gene (+- 2KB around TSS)
- 3 modification types (DNAm, K9, K27)

This 3D matrix is then converted into 58780 1-dimensional 12000 shape arrays (to be fed to ML models as single instances)

- 12000 is derived by concatenating the three 4000 long modification data into a single row of 12000

Stage 1: Use regression (tree-based) models to predict expected count (using mean squared error)

Model Type	MSE	RMSE
Random Forest	6106315.026	
XGBoost	8576452.711	
Gradient Boosting Regressor	6577097.108	
LightGBM Regressor	5931371.431	
CatBoost Regressor	6610849.514	
Support Vector Regressor	5542019.612	
AdaBoost Regressor	1527278184.653	

Comments:

- SVR saw best performance (potentially due to high-dimensional data)
- Followed by LightGBM (good for large datasets)
- Followed by Random Forest

Stage 2: Use classification (tree-based) models to predict 0 expected count or non-0 expected count (using precision, recall and F1)

Model Type	Accuracy	Precision	Recall	F1
Random Forest Classifier	0.7509	0.6692	0.6569	0.6630
XGBoost	0.7574	0.7261	0.5616	0.6333
LightGBM	0.7666	0.7317	0.5912	0.6540
Support Vector Machine	0.7726	0.7798	0.5442	0.6411

Comments:

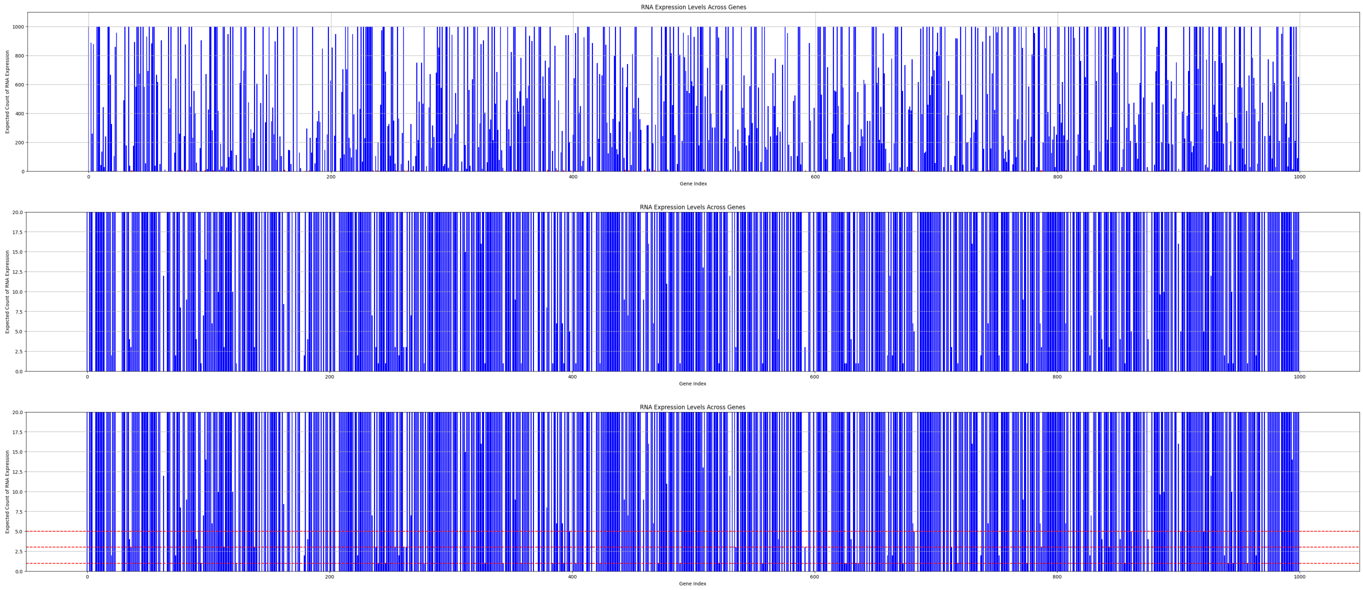
- More balanced prediciton of positive and negative classes for RF, followed by LightGBM with the second highest F1 score

- SVM saw the highest accuracy, with decent ability to predict positive class (non-silent genes) but poorer ability to predict negative class
- The same applies for XGBoost and LightGBM (although LightGBM is slightly better at predicting the negative class)
- potentially an ensemble model could see more accurate results?

Next steps:

- no need to standardise counts for regressor (would be more relevant if comparing between genes - making the assumption that measurement of noise is fair across the dataset)
- Would be helpful to see plot of expression against genome (expected count on one axis)
- Would be good to try a different threshold (slightly higher than 0 - use above plot to see where peaks are - will ultimately be an arbitrary choice but we assume that some thresholds would be better than others)

Plotting expression (to select a threshold for classification)



- selected 1000 genes and plotted expected counts against the index of that gene (and altered the ceiling to allow for a closer look at the lower level expression without altering the appearance of the visualisation, such as through the use of log)
- Will try thresholds 1, 3, and 5 and compare performance on the above default classification models

Comparison of thresholds

Threshold: 1

Model Type	Accuracy	Precision	Recall	F1
Random Forest Classifier	0.7815	0.6905	0.6229	0.6549
XGBoost	0.7774	0.7165	0.5482	0.6212
LightGBM	0.7908	0.7297	0.5904	0.6527
Support Vector Machine	0.7928	0.7617	0.5496	0.6385

Threshold: 3

Model Type	Accuracy	Precision	Recall	F1
Random Forest Classifier	0.8053	0.7232	0.5498	0.6247
XGBoost	0.8015	0.7117	0.5492	0.6200
LightGBM	0.8095	0.7215	0.5763	0.6408
Support Vector Machine	0.8119	0.7400	0.5579	0.6362

Threshold: 5

Model Type	Accuracy	Precision	Recall	F1
Random Forest Classifier	0.8037	0.7203	0.4767	0.5737
XGBoost	0.8093	0.7077	0.5313	0.6069
LightGBM	0.8187	0.7171	0.5712	0.6359
Support Vector Machine	0.8214	0.7307	0.5629	0.6359

Notes:

- Generally, it seems that increasing the threshold assists the model in predicting the positive class (non-silent) and makes it harder to predict the negative class (silent genes). Whilst accuracy and precision increase, recall decreases. Using the F1 harmonic mean may be more useful as a indicator of success considering our goals of classifying silent genes (as opposed to accuracy).
- Max F1 reached = RF classifier witha threshold of 0 (0.66)
- As we increase the threshold, we see a drop across all models in recall and F1, however, the drop for the LightGBM model is more slight than the others
- SVM performs best in classifying positive class but poorly in classifying negative class

It should be noted that the size of the classes is unbalanced with a greater number of silent genes than non-silent. The genes with low expression counts are close to our threshold and difficult to classify. To explore the impact of this, we will undersample the silent (negative) class.

Class sizes:

Threshold	Silent (negative class)	Non-silent (positive class)
0	36642	22138
1	39025	19755
3	41265	17515
5	42364	16416

Ensemble Model (SVM + LGBM):

- Was curious to see the impact of combining such models (SVM best at classifying positive class, LGBM most stable when classifying LGBM)
- Ensemble model combining lightgbm and SVM (with soft voting)
- threshold = 0
- Unbalanced classes
- Based on the below results, the combination of these models didn't provide a significant increase to F1 (as compared to the standalone LGBM or SVM F1 values)

Accuracy	Precision	Recall	F1
0.7734	0.7567	0.5787	0.6558

Repeating models with balanced classes

Due to the imbalance between silent and non-silent genes, we will repeat the classification task using a threshold of 0 and undersampling the silent class. Both classes are now the same sized (matched to the size of the smallest class = 22138 & 22138)

Model Type	Accuracy	Precision	Recall	F1
Random Forest Classifier	0.7185	0.7164	0.7260	0.7211
XGBoost	0.7243	0.7617	0.6550	0.7043
LightGBM	0.7293	0.7524	0.6858	0.7176
Support Vector Machine	0.7377	0.8029	0.6320	0.7072

Notes:

- F1 scores have increased considerably (around 5% each)
- Accuracy has decreased but was likely inflated previously due to the imbalance of class sizes
- Precision and recall have both increased
- Using the same number of rows for silenced and unsilenced
- SVM best for precision but still not great at classifying silent genes

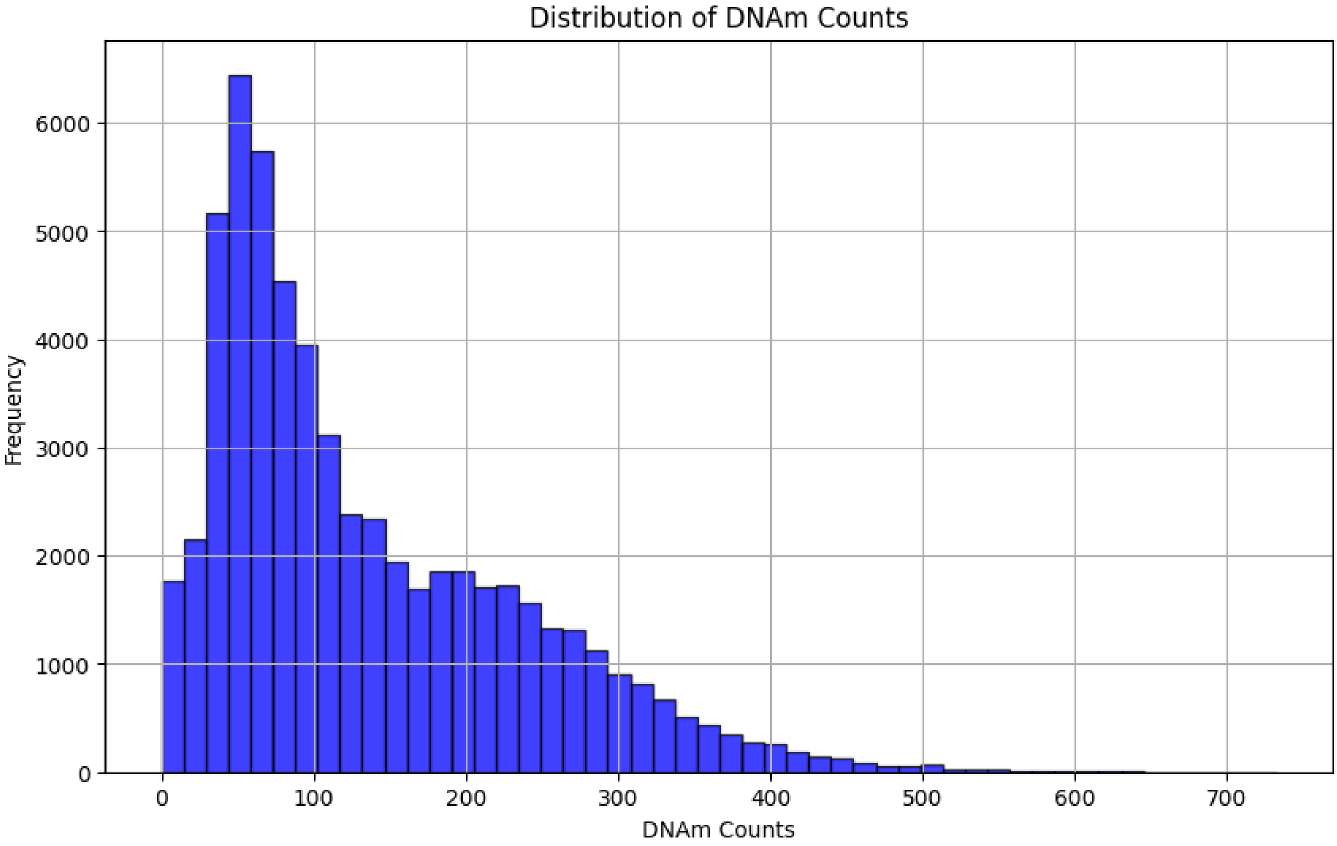
Modelling with DNAm as output

To explore the interplay between histone modifications, expression and DNA methylation, we will model using expression and histone modifications as our input and DNA methylation as our output. To begin with more simple and interpretable modelling, we will start with similar ML models.

NOTE: predicting DNAm is not as straightforward as expression (with a count value that can be classified as silent or non-silent). To begin, I attempted a multioutput model (predicting binary output for all 4000 positions) but am unsure whether this is appropriate. Regardless, I will be meeting with HPC team to assist with running a script using distributed computation.

For a simpler approach, we predict the count of methylated bases (x/4000).

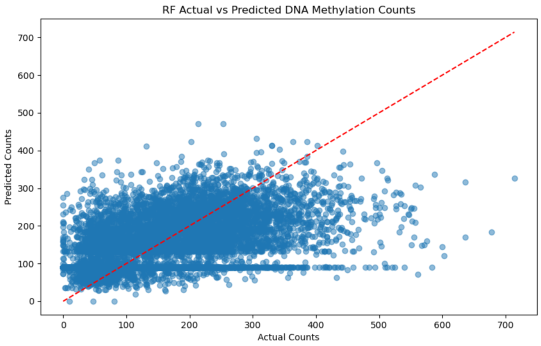
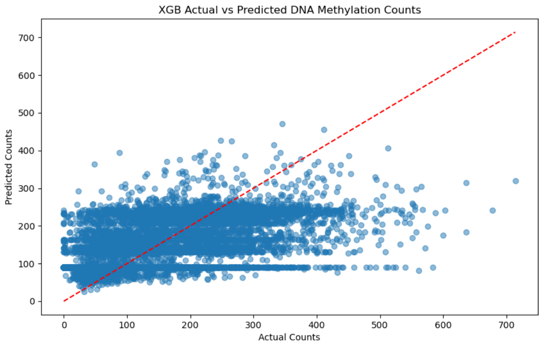
The distribution of the DNAm counts is shown below:

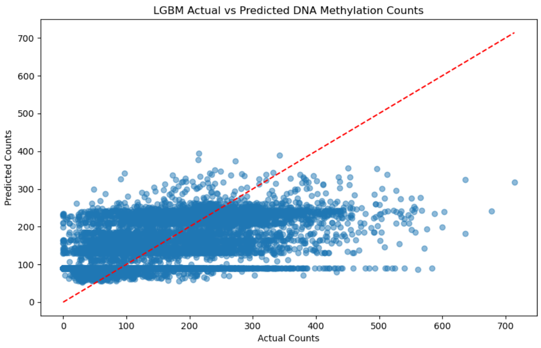
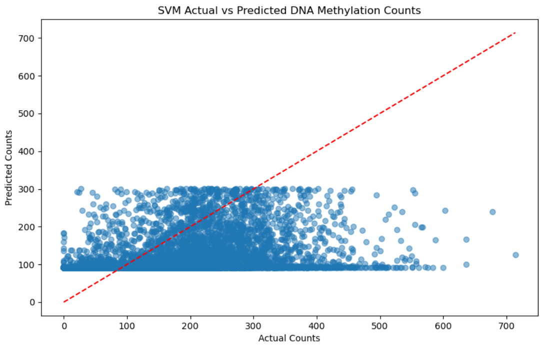


Regression Results

Model	**MAE**	**MSE**	**R^2**
Random Forest	62.3022	7097.7616	0.3122
XGBoost	60.4960	6689.7580	0.3518
LightGBM	60.1549	6581.1190	0.3623
SVM	68.9159	9374.4317	0.0916

To assist in the interpretation of these results, actual vs predicted graphs are provided below:

Random Forest	**XGBoost**
	
LightGBM	**SVM**

LightGBM	SVM
	

Notes:

- LightGBM sees lowest error
- Somewhat difficult to interpret error
- Distribution of the DNAm counts is skewed - should I be considering transforming count by taking the log?
- Based on actual vs predicted plots, seems to sit consistently around 100 - 300 for predicted values. Could this be improved with transformations?

Exploring the use of neural networks

Simple (single) fully connected layer

Using a single fully connected linear layer and cross entropy loss. Using:

- 0 threshold
- Linear layer
- Balanced and unbalanced class (as listed)
- Criterion = nn.CrossEntropyLoss()
- Optimizer = optim.Adam(model.parameters(), lr=0.001)
- Epochs = 10
- batch norm for regularisation (only in stated table)

Balanced classes

Accuracy	Precision	Recall	F1
0.5854	0.5528	0.9050	0.6863

Unbalanced classes

Accuracy	Precision	Recall	F1
0.6857	0.5798	0.5720	0.5759

Balanced classes & Batch Normaslisation

Accuracy	Precision	Recall	F1
0.7245	0.7622	0.6547	0.7044

Notes:

- Achieving very high recall with balanced classes (great at classifying silent genes but poorer performance classifying non-silent genes)
  - Opposite effect of using the simpler ML models
- Including batch normalisation balances precision and recall (+ highest F1 score)
- F1 score is comparable to the balanced simpler ML models (RF, LGBM, XGB, SVM) and is only utiltising a single linear layer
  - Promising results considering its simplicity