Machine Learning Uncovers CCM Isoforms as Transcription Factors

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Supplemental Materials

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Methodology

Our methodological strategy deploys a two-step procedure, first employing the Evolutionary Scale Modeling (ESM), a sophisticated protein Large Language Model (LLM), for the representation of protein sequences. Subsequently, we use a cost-sensitive Support Vector Machine (SVM) classifier to facilitate a efficient prediction of transcription factors.

Evolutionary Scale Modeling (ESM): The ESM is an advanced transformer-based LLM designed specifically for proteins, trained on a vast corpus of 250 million protein sequences [1]. The primary goal of this training was to enable the model to predict structure, function, and various protein properties directly from individual sequences through masked language modeling. Owing to its flexibility, ESM is perfectly suited for fine-tuning a multitude of tasks that require protein sequences as input. The core of our methodology lies in utilizing ESM to generate highly representative encodings of protein sequences that are subsequently fed to our prediction model. In this study, we utilize ESM-2, a state-of-the-art protein language model that outperforms all tested single-sequence protein language models across a range of structure prediction tasks and enables atomic resolution structure prediction [2]. It offers unprecedented capabilities in understanding and predicting protein interactions and functions.

Cost-sensitive SVM: SVMs are robust classifiers that aim to identify an optimal hyperplane that differentiates between classes within the feature space. Traditional SVMs may perform suboptimally on imbalanced datasets due to their lack of consideration for the differential costs associated with the misclassification of minority class examples. We address this issue by

employing a cost-sensitive SVM approach, which assigns the misclassification cost for each class to be inversely proportional to its frequency, thus ensuring fairer treatment of minority classes [3].

Experimental Setup and Data Preprocessing

We conduct our experiments using a 5-fold cross-validation strategy on a dataset consisting of 4330 protein sequences, with a distribution of 3315 Non-Transcription Factor (NTF) samples and 1015 Transcription Factor (TF) samples. To maintain uniformity across sequences, all are truncated or padded to achieve a standardized length of 1000 amino acid residues. The application of 5-fold cross-validation yielded an average macro F1 score of 0.9505, specificity of 0.9625, sensitivity of 0.9655, and balanced accuracy of 0.9640.

Suppl. Figure 1: **Multi-model machine learning utilized to predict functionality of protein isoforms**. **A.** Convolution Neural Network (CNN) with a threshold of 0.5 found a possible transcription factor in 60% of the testing repetitions. This result was consistent as it was the same sequence repeated in each of the positive repetitions **B.** CNN with a lower threshold: The unequal balance of transcription factors to non-transcription factors led the research team to lower the threshold to 0.1 to increase the sensitivity to the unbalanced nature of transcription factors to non-transcription factors. This resulted in more potential transcription factors being discovered in all repetitions of testing. **C.** A second model of machine learning was utilized in the form of Biased-SVM models. The research team repeated the process of starting with a threshold of 0.5 to predict any potential transcription factors and found multiple (n=8) in one testing repetition. **D.** Following the same procedure as the CNN the threshold for the biased-svm model had the threshold reduced to 0.1 to induce in-balance that is found in the protein-transcription ratio found in nature.

Suppl. Figure 2: **Multiple machine learning models found concurrent and unique proteins predicted to be transcription factors in multiple repetitions.** A Venn Diagram was utilized to compare the sequences found between the two models. The CNN and biased SVM models predicted 7 concurrent sequences to function as transcription factors, while each predicted their own unique sequences to also function as transcription factors.

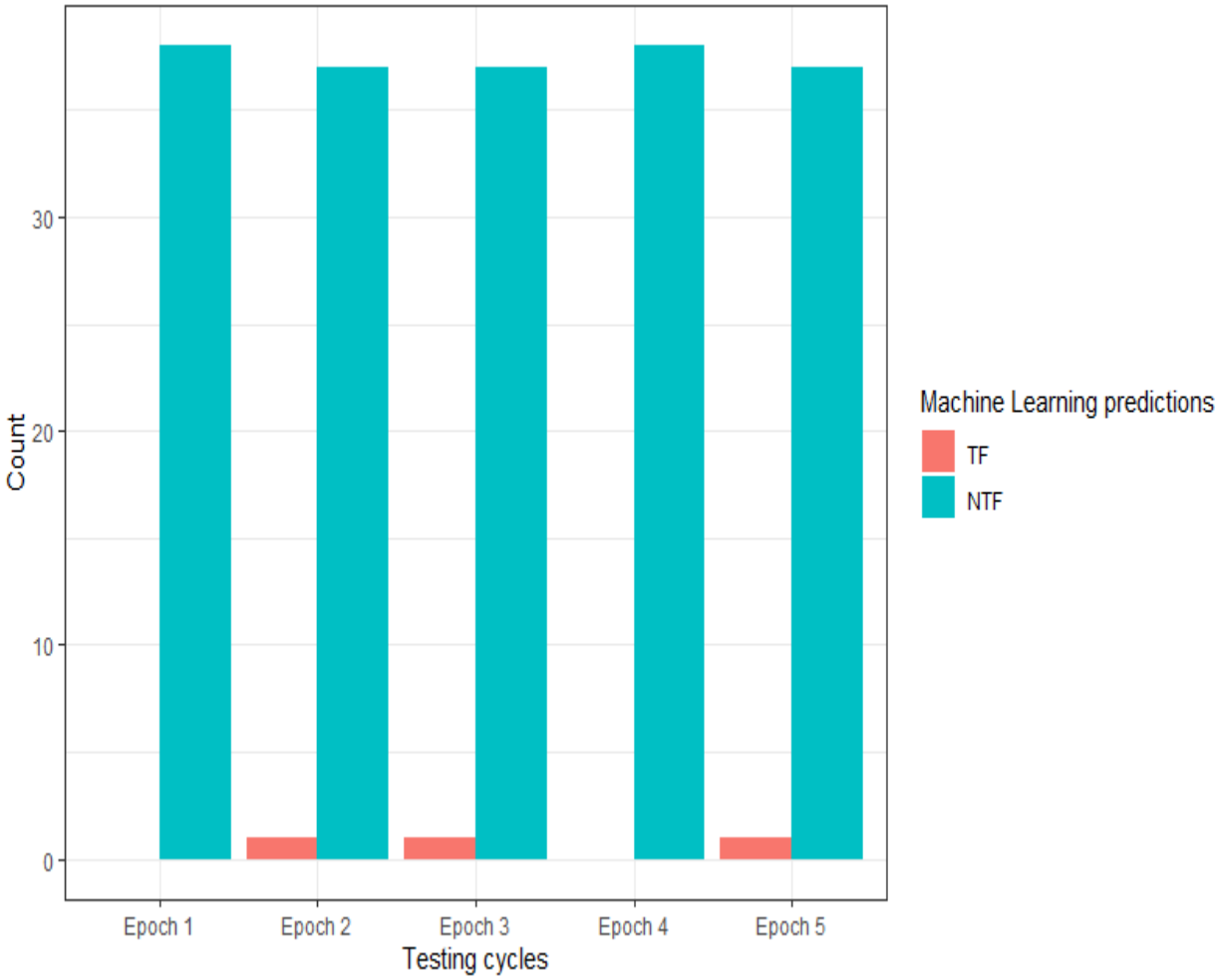
Suppl. Table 1: 5-Fold Cross Validations results of the CNN model used to test the sequence on a unique training model of 4331 unique FASTA sequences. The research team set a standard for all validations results to be at 95% to minimize all type 1 and type 2 errors. While the CNN did not deliver the F1 score of 95% we compared the results to the Biased-SVM as a way to verify findings.

Suppl. Table 2: 5-Fold Cross Validations results of the biased-SVM model used to test the sequence on a unique training model of 4331 unique FASTA sequences. Where the CNN model was lacking the 95% in all categories the biased-SVM model was able to accomplish this. We used the CNN model as a secondary comparison to the primary results found by the biased-SVM model to prevent any type 1 or type 2 errors.

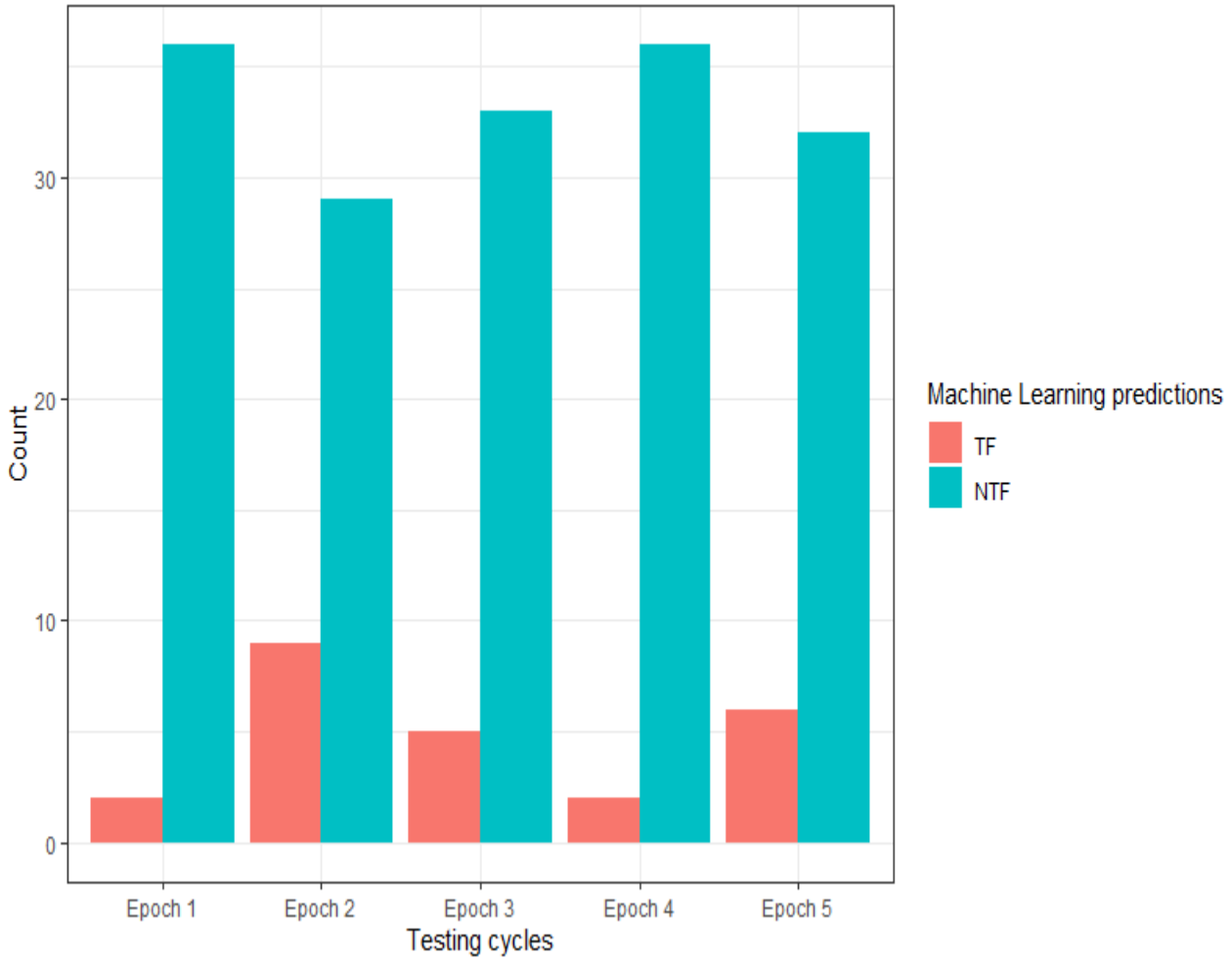
Other Suppl. Table containing all raw data for ML calculation, and will be provide by PI under the request.

Suppl. Figure 1:

1A: CNN Threshold ≥ 0.5

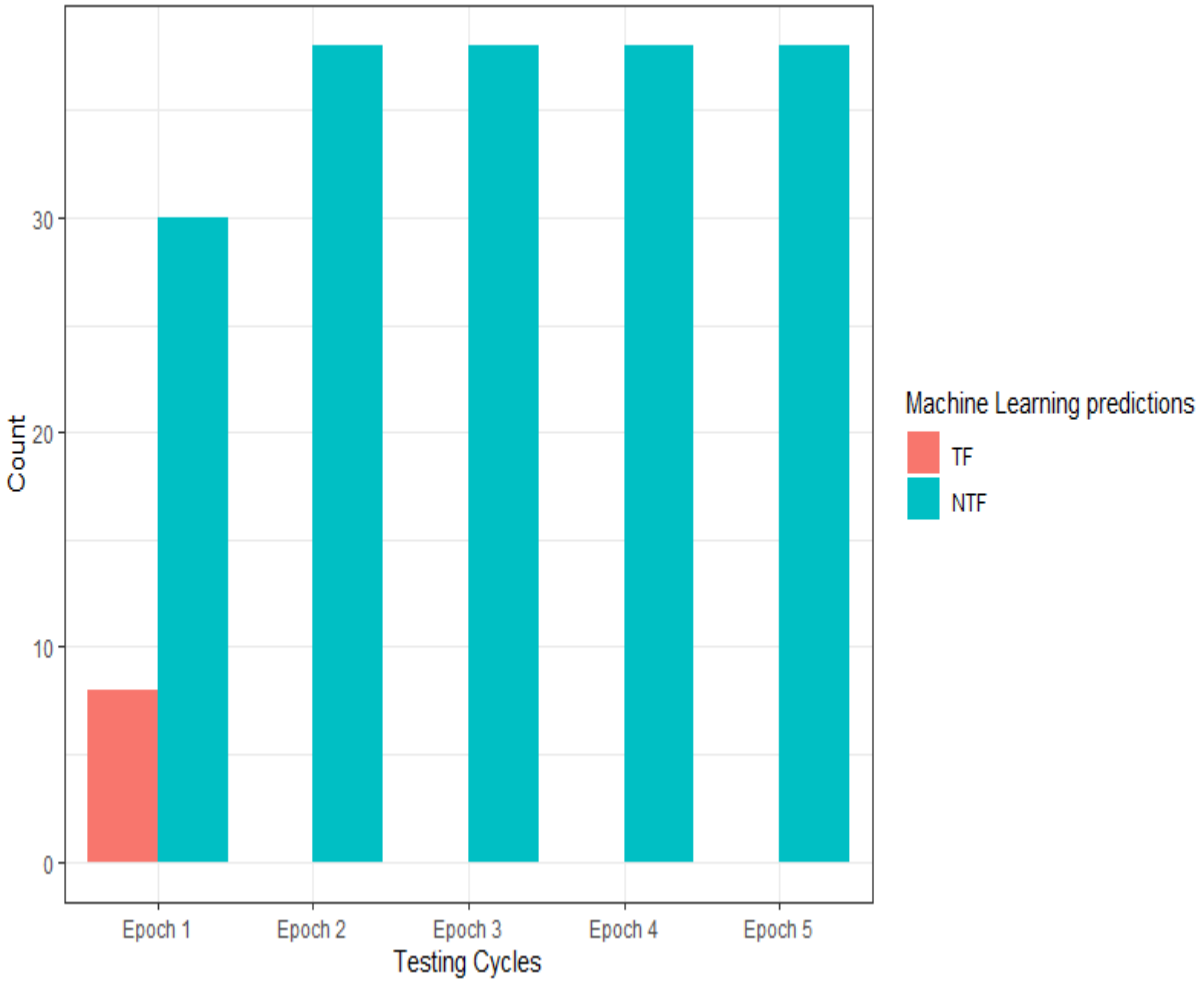


1B: CNN Threshold ≥ 0.1

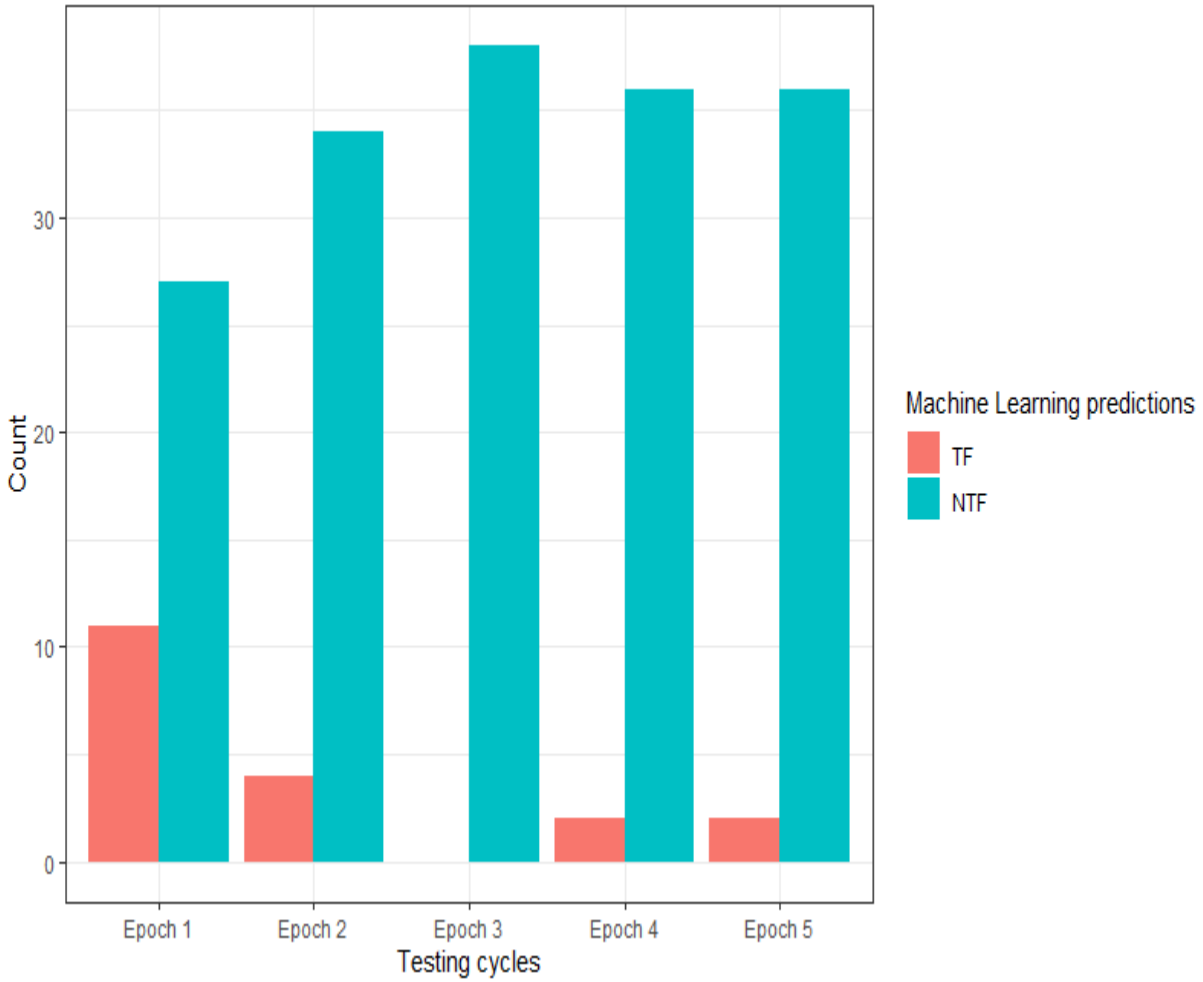


Suppl. Figure 1:

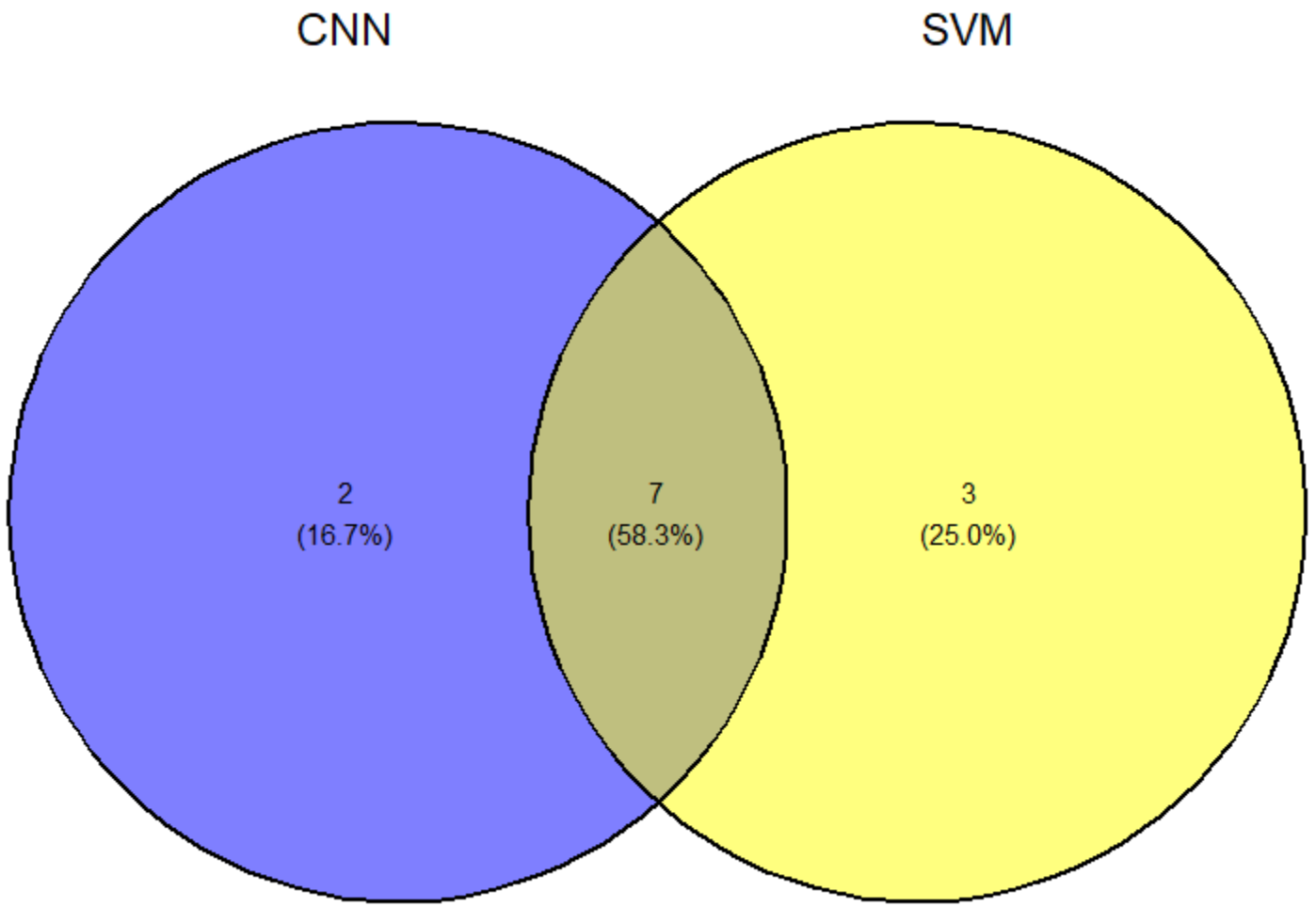
1C. SVM Threshold ≥ 0.5



1D: SVM Threshold ≥ 0.1



Suppl. Figure 2



Venn diagram of mutual and unique predictions per learning model technique

Suppl. Table 1: 5 Fold Cross Validation results of CNN model

CNN 5-Fold Cross validation results	
F1 Score	0.940
Specificity	0.956
Sensitivity	0.954
Balanced Accuracy	0.955

Suppl. Table 2: 5 Fold Cross Validation results of Biased-SVM model

Biased-SVM 5-Fold Cross Validation	
F1 Score	0.950
Specificity	0.962
Sensitivity	0.966
Balanced Accuracy	0.964