

华中科技大学

生物信息数据挖掘报告

Prediction of Splicing Sites by WAM

授课教师 : 周艳红
课程名字 : 生物信息数据挖掘
班 级 : 生信基地1801
姓 名 : 苏济雄
学 号 : U201812416
日 期 : 2021.06.06

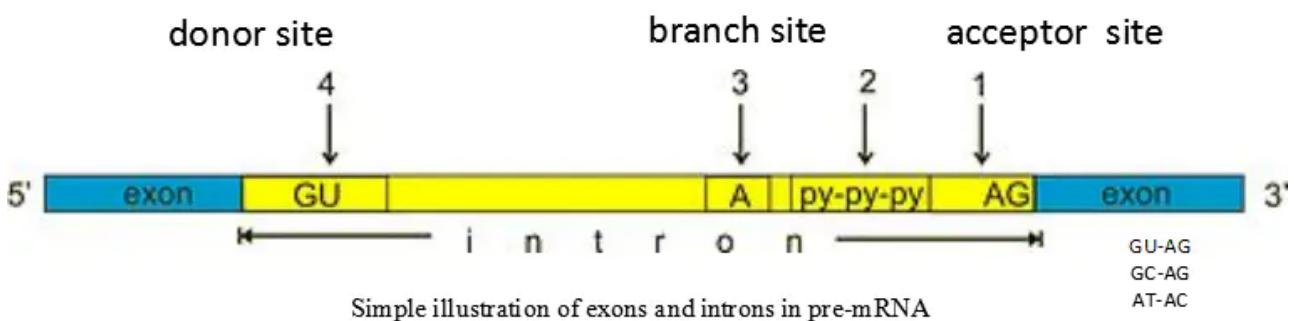
Prediction of Splicing Sites by WAM

1 AIM:

- 充分理解 WAM 数学原理，能够构建 WAM 模型进行 donor 位点预测；
- 学会使用编程语言提取序列数据相关信息；
- 学会模型优化以及模型预测性能评估；

2 HYPOTHESIS:

真核生物的基因包含外显子和内含子，剪接时需要区分外显子和内含子，研究发现在内含子和外显子边界存在保守的剪切位点，包括内含子 5' 和 3' 末端序列及中间分支点（branch site）附近序列。5' 剪切点称为供体点（donor site），3' 剪切点称为受体点（acceptor site）。内含子开始和末尾的碱基最为保守，为 GU-AG（约占 99.24%），少数为 GC-AG（0.7%），极少数为（AU-AC）。



内含子 donor site 和 acceptor site 上下游的碱基位点与其他位点碱基分布存在差异并假设相邻的碱基存在关联，通过碱基的分布差异可以区别真剪切位点和假剪切位点。在构建模型时，选取的 donor site 上下游序列的长度将极大影响着模型预测性能，需要选取最适长度进行模型构建。

3 METHODS:

3.1 Description of models

权重矩阵模型 WAM 是以贝叶斯公式为核心原理，考虑 donor 位点上下游相邻碱基的相关性。通过提取剪切位点上下游一定长度的信号序列，计算其频率分布，建立判别函数。通过给输入的序列打分，若分值超过设定的阈值，则预测该序列含有 donor site，反之，则判定为其他序列。

3.2 Program design

3.2.1 第一步：数据清洗

Training Set 文件夹有 462 个文件，每个文件对应一个基因，共 2381 个内含子，即 2381 个 donor sites 和 acceptor sites，所有文件的后缀名都是"TXT"

- 第一行如"LOCUS AB000381 35863 bp DNA PRI 14-MAY-1997"，提取的时候需要注意每条信息以多个空格分割。
- 第二行如"CDSjoin(28199..28271,28881..28988,34291..34586)"，显示外显子的位置。需要注意每个基因的第一个外显子无 acceptor site，最后一个外显子无 donor site。
- 第三行起为基因序列，以每行 60 个间隔，每个基因序列都是小写

Testing Set 文件夹有 570 个文件夹，共 2079 个内含子，该文件夹下的文件名后缀有 TXT 和 txt 混存，与 Training Set 相比有些结构区别：

- 第一行只有">+ 基因座，如">ACU08131"

- 第二行为"> 基因座名"+ 外显子位置, 如">AGGGLINE(3066..3157,3281..3503,4393..4521)"
- 第三行起为基因序列, 每行 60 个, 基因序列为大写。

两个文件夹内的基因序列除了 ACGT 正常碱基外, 还存在未知碱基。

3.2.2 第二步: signal 序列的提取

利用数据集中提供的 CDSjoin 提取训练集和测试集的 donor site 上下游一定长度的序列, 保存为列表。

并提取训练集中在对应位置含有 GT 碱基对但不是 donor site 的序列, 设为假 donor signal 序列。

3.2.3 第三步: 概率矩阵的构建

1. 碱基概率分布矩阵: 分别计算 donor signal 和 pseudo donor signal 每个位置出现四种碱基 P 的概率, 构建分布概率矩阵。

$$P(i, x) = \frac{f(i, x)}{\sum f(i, x)}$$

2. 条件概率分布矩阵: 考虑到碱基之间的分布不是绝对独立的, 所以计算出现前一个碱基出现下一个碱基的条件概率。得到相邻碱基的条件概率分布矩阵。

$$P(i, x, y) = \frac{f(i, x, y)}{f(i - 1, x)}$$

3.2.4 第四步: 进行预测

1. 通过决策函数, 先计算训练集提取出的信号序列的分值, 统计正样本和负样本的分值分布情况
2. 提取测试集每个文件中的 donor 序列和假 signal 序列, 放在一个文件中, donor 序列标记为 1, 假 signal 序列标记为 0, 给测试集每个 signal 序列打分

判别函数:

$$S(X) = \ln \frac{P^+(X)}{P^-(X)} = \ln \frac{p^+(1, x_1)}{p^-(1, x_1)} + \sum_{i=2}^{\lambda} \ln \frac{p^+(1, x_{i-1}, x_i)}{p^-(1, x_{i-1}, x_i)}$$

3.2.5 第五步: 评估模型效果

根据每个序列的分值, 做 ROC 和 PR 图, 通过阈值的变化, 查看 Recall 和 Precision 的曲线变化

常见的评估模型指标

$$\begin{aligned} Sn/Recall &= \frac{TP}{TP + FN} \\ Precision &= \frac{TP}{TP + FP} \\ Sp &= \frac{TN}{TN + FP} \\ FDR &= 1 - Sp = \frac{FP}{TN + FP} \\ Accuracy &= \frac{TP + TN}{TP + TN + FN + FP} \\ F1-score &= 2 * \frac{precision * recall}{precision + recall} \end{aligned}$$

4 RESULTS

4.1 统计剪切位点上下游碱基的分布情况

为了解剪切位点上下游碱基的分布情况，统计 donor site 和 acceptor site 附近的序列共 60 个碱基，可以看到 donor site GT 碱基对下游 3 个碱基和上游 4 个碱基具有较强的特征性。acceptor site 则是 AG 上游有较长的连续 C/T 信号。

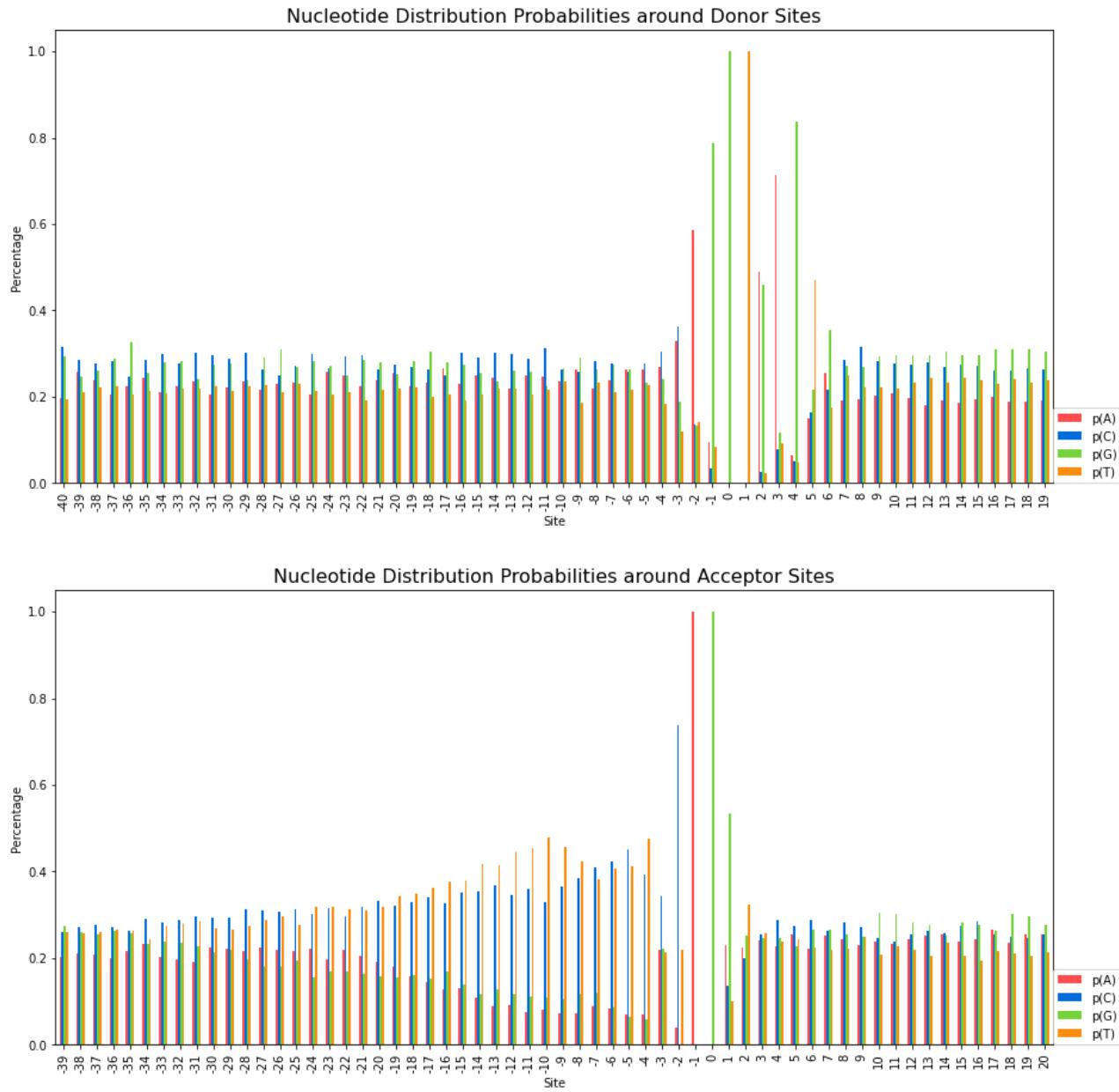


图1. donor site 和 acceptor site 附近的序列共 60 个碱基的weblogo图

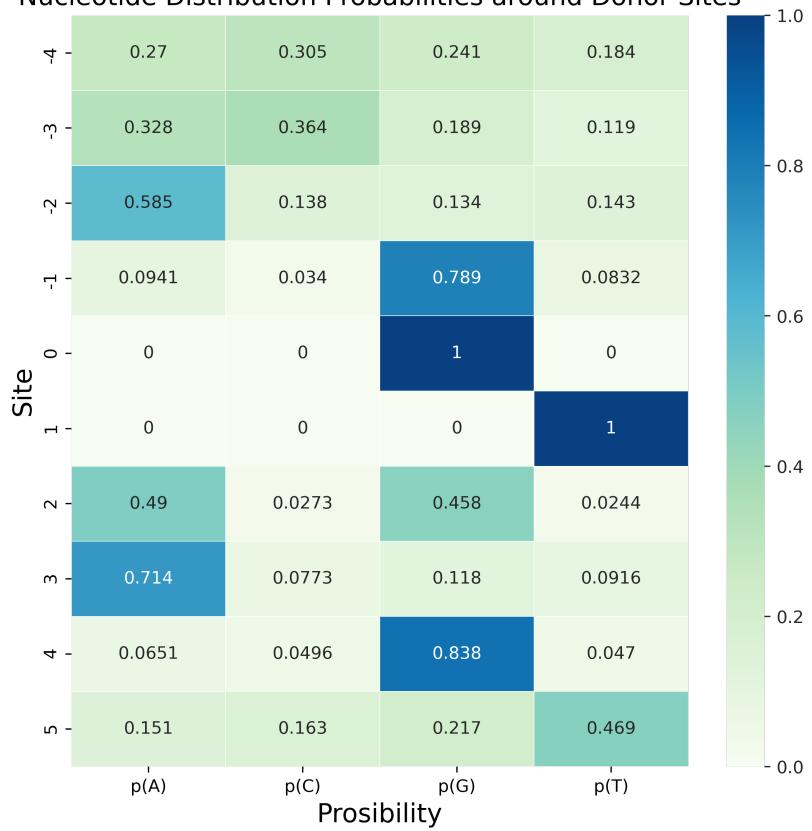
提取donor site signal 信号的时候，先选择 donor site 外显子和内含子边界上游4个碱基和下游6个碱基。

4.2 碱基概率分布矩阵和条件概率分布矩阵

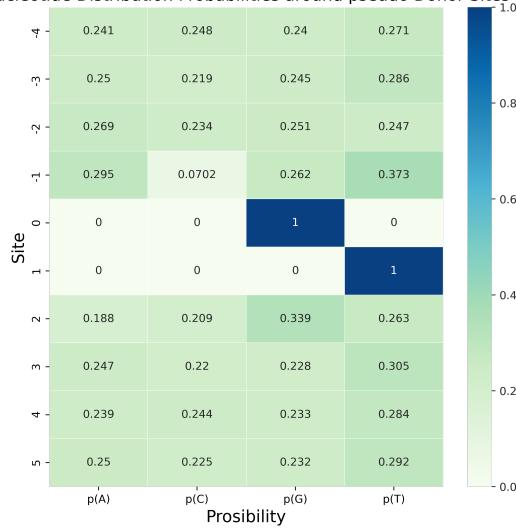
根据提取的 signal 序列，得到碱基概率分布矩阵和条件概率分布矩阵。

从分布热图来看，可以看到 donor signal 和 common signal、pseudo donor signal 的碱基概率分布矩阵和相邻碱基条件概率矩阵存在较大差异。对于普通位点来说，每个位点的碱基分布十分相近。pseudo donor signal 除了 GT 碱基对，其他位置的碱基概率分布与 true donor signal 存在明显的区别。

Nucleotide Distribution Probabilities around Donor Sites



Nucleotide Distribution Probabilities around pseudo Donor Sites



Nucleotide Distribution Probabilities around non Donor Sites

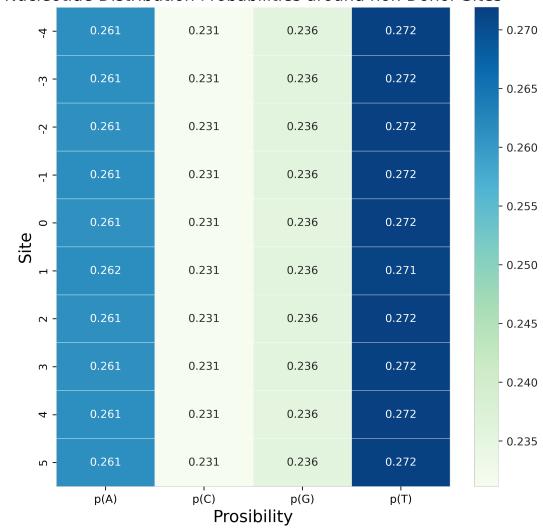


图 2. donor site, pseudo donor site和common site的碱基分布概率矩阵

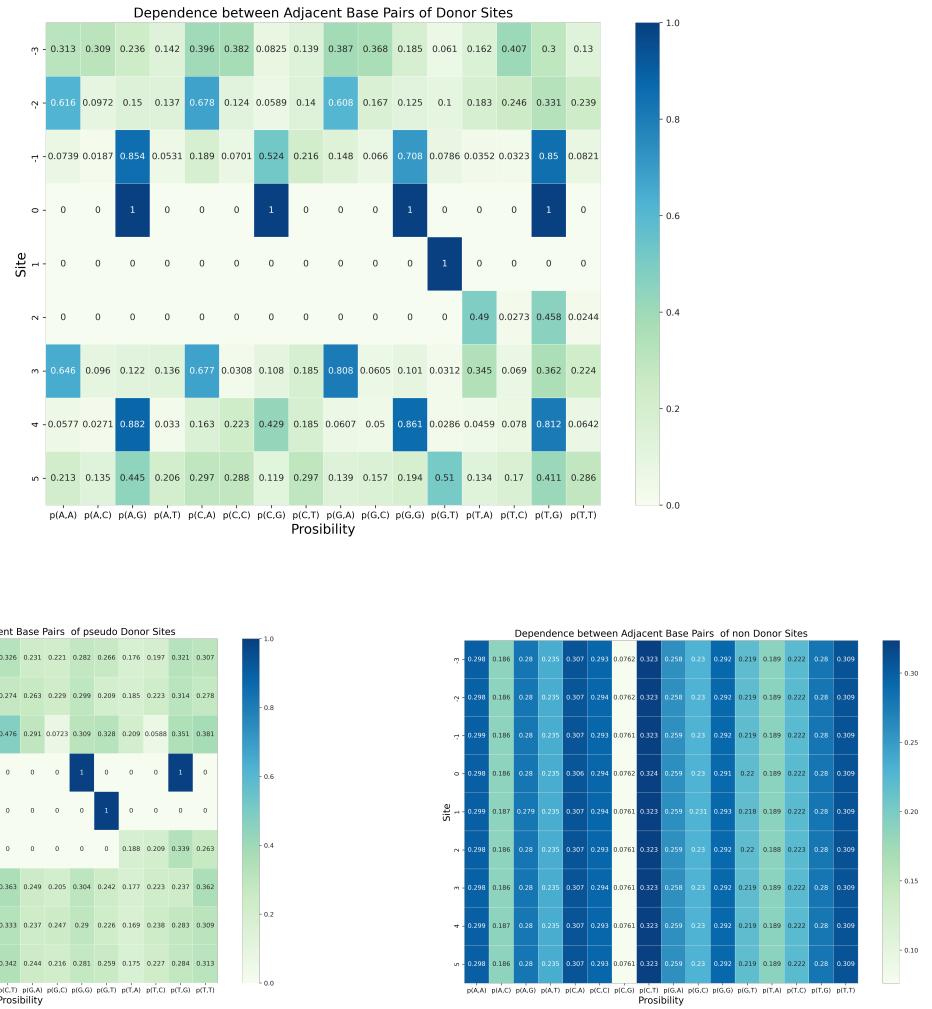


图 3. donor site, pseudo donor site和common site的相邻碱基条件概率矩阵

4.3 WAM 得分情况

根据 WAM 判别函数计算提取的训练集所有 signal 序列得分，并统计其分布情况。

绿色代表 true donor signal，红色代表 pseudo donor signal，查看正样本和负样本的分值分布情况。正样本的分值绝大多数分布在 0 到 6 之间，而负样本的分值大多数分布在 0 到 -8 间，但还有一部分分值高于 0。通过分值分布的累计图，可以大概估计其 recall 和 precision 值。

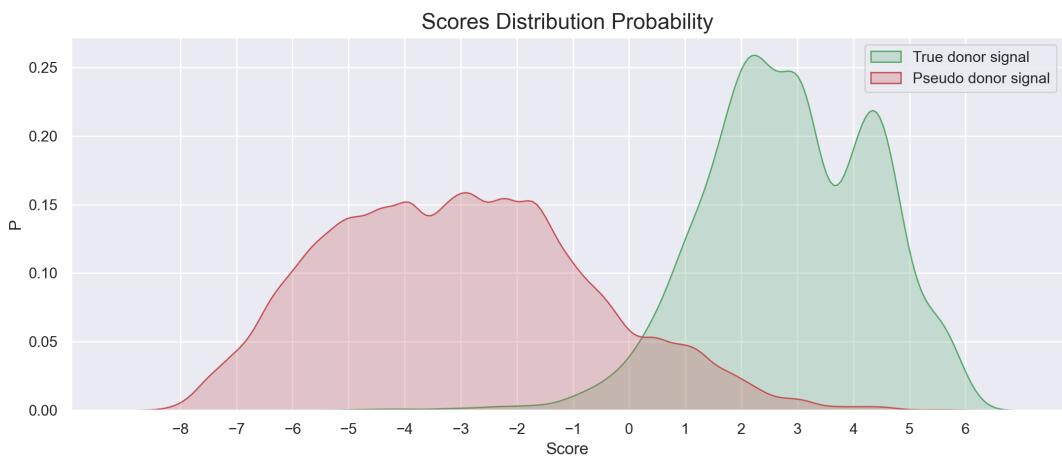


图 4. 训练集真样本和负样本的得分分布

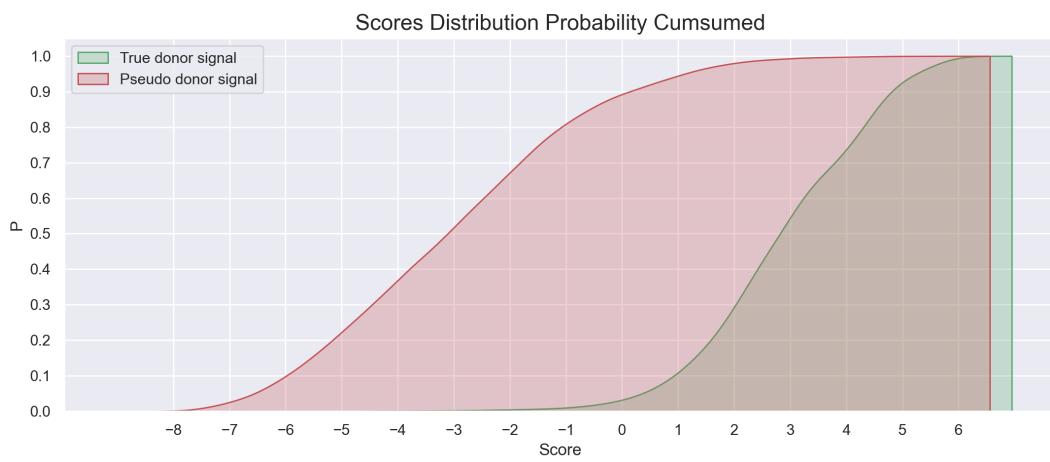


图 5. 训练集真样本和负样本的得分累计分布

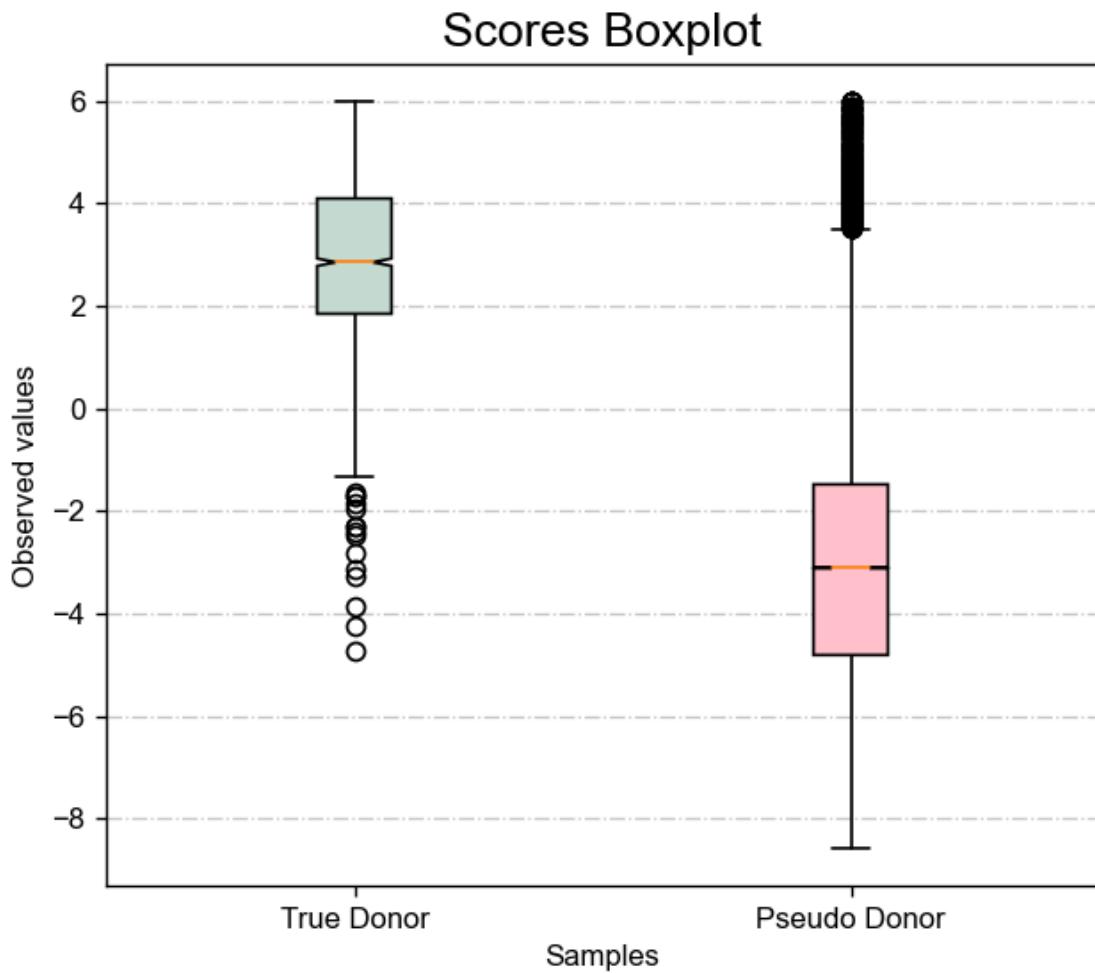


图 6. 训练集真样本和负样本的得分分布箱式图

4.4 预测及评估模型

通过调整 donor 位点上下游碱基序列长度，比较不同滑动窗口的预测性能

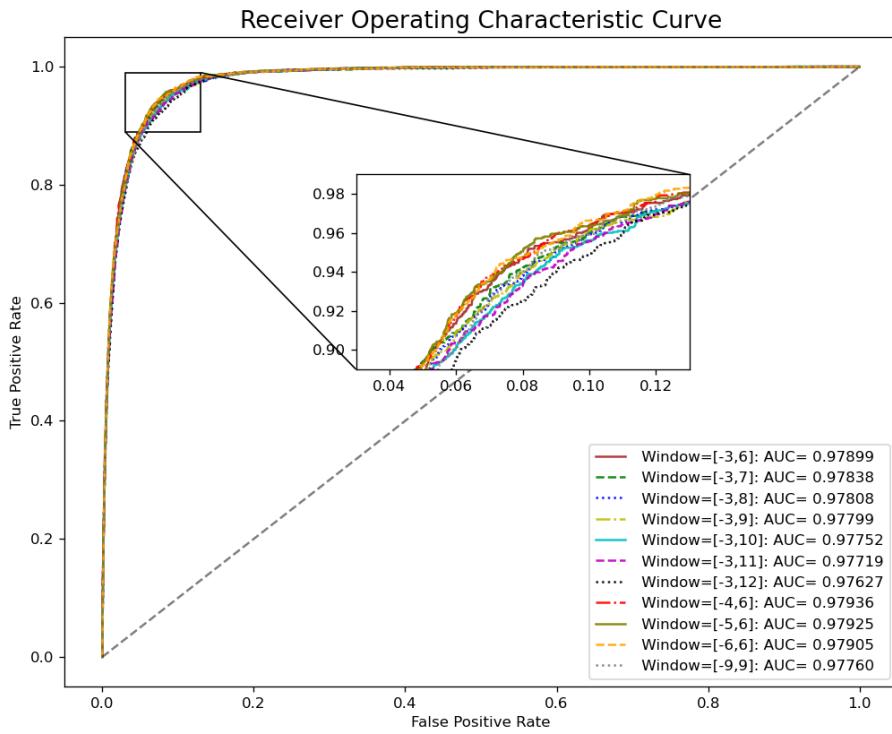


图7. 不同signal序列的ROC曲线

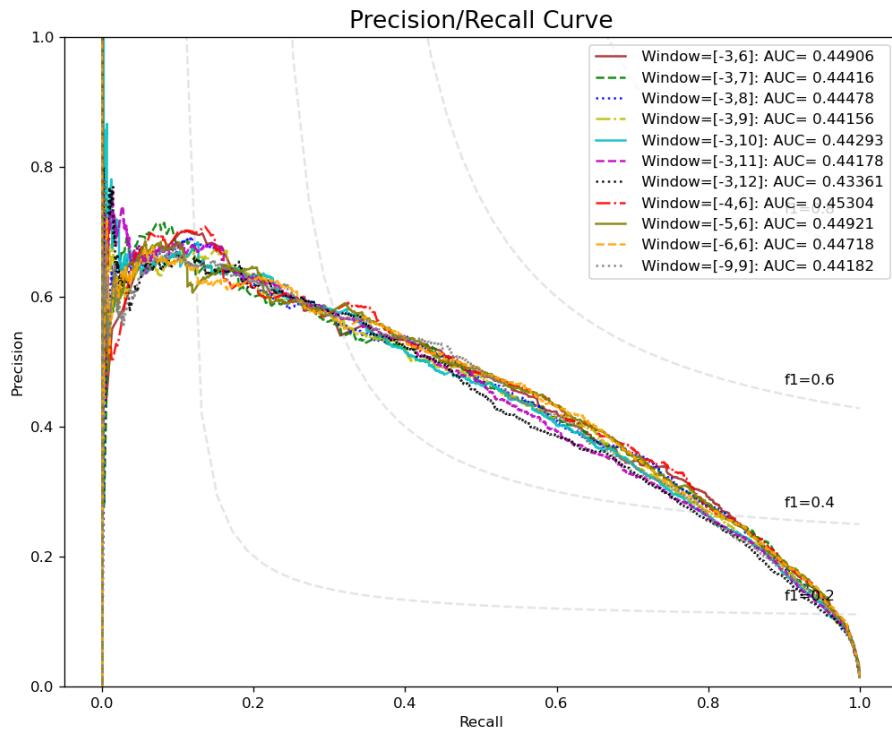


图8. 不同signal序列的ROC曲线

可以看到window=[-4,6]时，AUROC为0.97936，AUPRC为0.45304，在检测的所有signal序列长度中，性能最好。

分析window=[-4,6]时，WAM和WMM的性能差异，画ROC图和PR图，当阈值为0.2750的时候，为ROC图的最佳临界点，这时Sn-FPR的值取最高，Recall达到0.96且误判率较低，Precision也达到0.13，F1-score为0.23。WAM的ROC曲线和PR曲线都显著高于WMM模型。

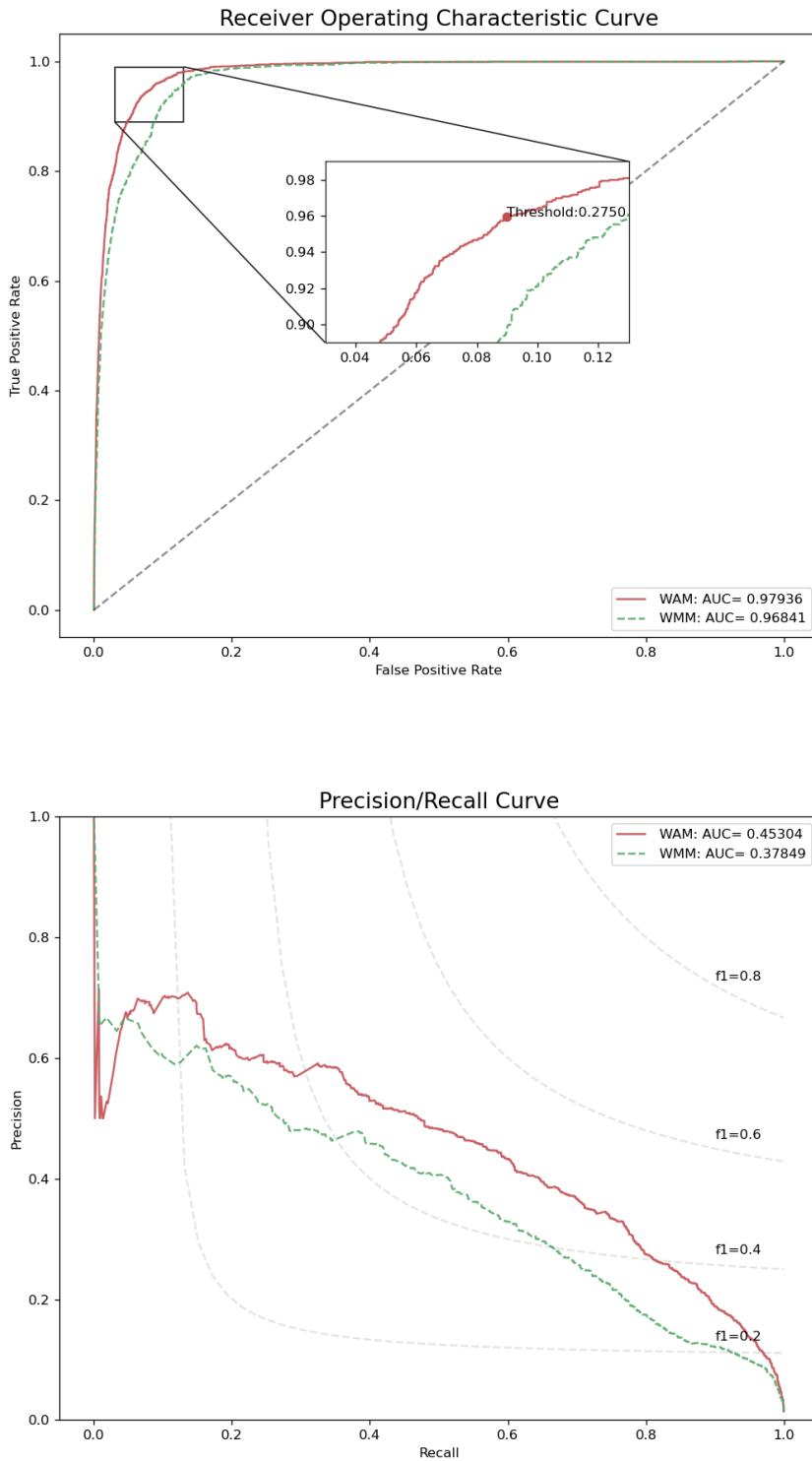


图 9. window=[-4,6]时，WAM和WMM 预测性能比较

接着绘制window=[-4,6]时，各阈值下Precision、Recall和F1-score变化的趋势图。随着阈值提高，Precision先上升，但不可避免的Recall值不断下降，在阈值为5.0左右，Precision又回落。在阈值为2.7时，F1-score取得最大。F1-score是综合考虑了Precision和Recall值的指标。

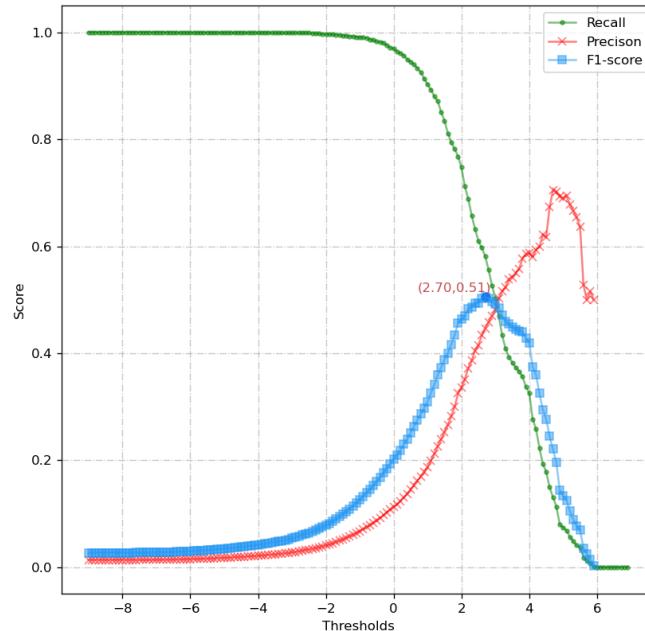


图 10. window=[-4,6]时，WAM各阈值下的Precision和Recall、F1-score趋势图
表1 各阈值的预测结果

Threshold	TP	FP	TN	FN	Recall	Precision	F1-Score	Sn
-9	2079	149183	0	0	1	0.013744	0.027116	0
-6	2078	134741	14442	1	0.999519	0.015188	0.029921	0.096807
-3	2077	72629	76554	2	0.999038	0.027802	0.054099	0.513155
-1	2060	28518	120665	19	0.990861	0.067369	0.12616	0.808839
0	2016	15820	133363	63	0.969697	0.11303	0.20246	0.893956
1	1877	8166	141017	202	0.902838	0.186896	0.309685	0.945262
1.5	1735	5120	144063	344	0.834536	0.2531	0.388404	0.96568
2	1554	3069	146114	525	0.747475	0.336145	0.463742	0.979428
2.5	1268	1785	147398	811	0.609909	0.415329	0.494154	0.988035
3	1045	1126	148057	1034	0.502646	0.481345	0.491765	0.992452
3.5	796	673	148510	1283	0.382876	0.541865	0.448703	0.995489
4	677	475	148708	1402	0.325637	0.587674	0.419065	0.996816
4.5	371	230	148953	1708	0.178451	0.617304	0.276866	0.998458
5	154	69	149114	1925	0.074074	0.690583	0.133797	0.999537
5.5	77	44	149139	2002	0.037037	0.636364	0.07	0.999705
6	0	0	149183	2079	0	NA	NA	1

绘制阈值为 0.2750 (ROC 最佳参数点) 和阈值为 2.7 (F1-score 最高点) 的混淆矩阵，以比较两个最佳参数点的区别。ROC最佳参数点是真阳性率减去假阳性率最大的阈值点，代表分类模型发现正样本和负样本的总能力。如图 11，可以看此时Recall=0.96, Precision=0.13, F1-score=0.23，但从混淆矩阵可知这时FP多达13415。而F1-score是精确率和召回率的调和平均数，认为召回率和精确率同等重要，最高点则是综合考虑Precision和Recall值，从图11右侧的混淆矩阵得知，此时Precision=0.45, Recall = 0.58, F1-score=0.51，虽然FP减少到了1498，但TP也只有1209。

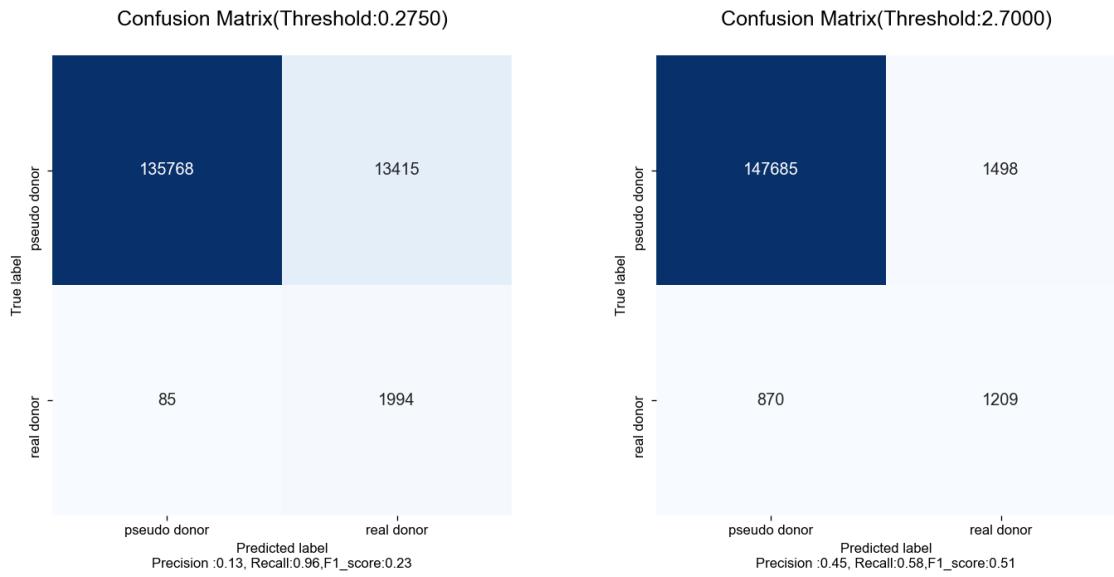


图 11. 混淆矩阵：左，ROC最佳参数点的混淆矩阵；右，F1-Score最高时的混淆矩阵

4.5 Double-WAM

之后我尝试了使用两次 WAM，尝试提高模型的预测准确度。整体策略是：

第一层 WAM 使用所有正负样本来训练模型，将分值小于阈值的训练集中正负样本序列过滤掉。

第二层 WAM 使用所有第一层 WAM 打分分值高于阈值的样本，再次进行训练。

在对测试集进行预估的时候，也是先用第一层 WAM 进行打分，分值小于阈值的样本判定为负样本，分值高于阈值的样本再送入第二层 WAM，再次进行打分。最终设定阈值为 -4，这样第一层就能过滤掉很大部分的负样本，同时绝大部分正样本分值都高于这个阈值。

从第二层 WAM 对训练集输入的正负样本的打分分布情况来看，正样本和负样本并不能很好区分，在 -3 和 4 分值区间内都有所重叠。虽然较于第一层 WAM 有所改善。

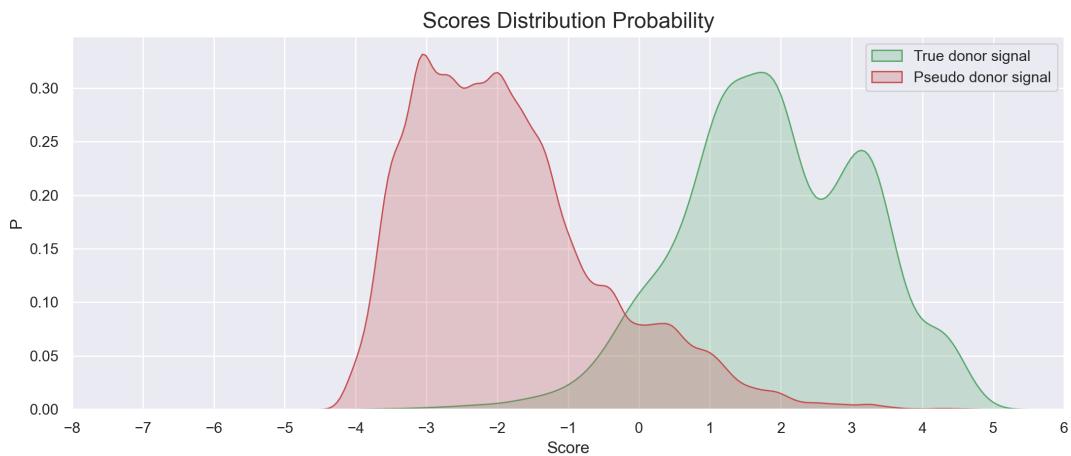


图 12. 第二层 WAM 对训练集输入的正负样本的打分分布情况

从 ROC 图和 PR 图可以得知，Double-WAM 的 ROC 和 PR 曲线都略微有点提升，说明两层的 WAM 确实能稍微提高模型预测的准确度，但效果比较有限。鉴于第二层的 WAM 依然对输入的正负样本的区分度并不好，可能需要更换其他模型来识别这些难以区分的样本。

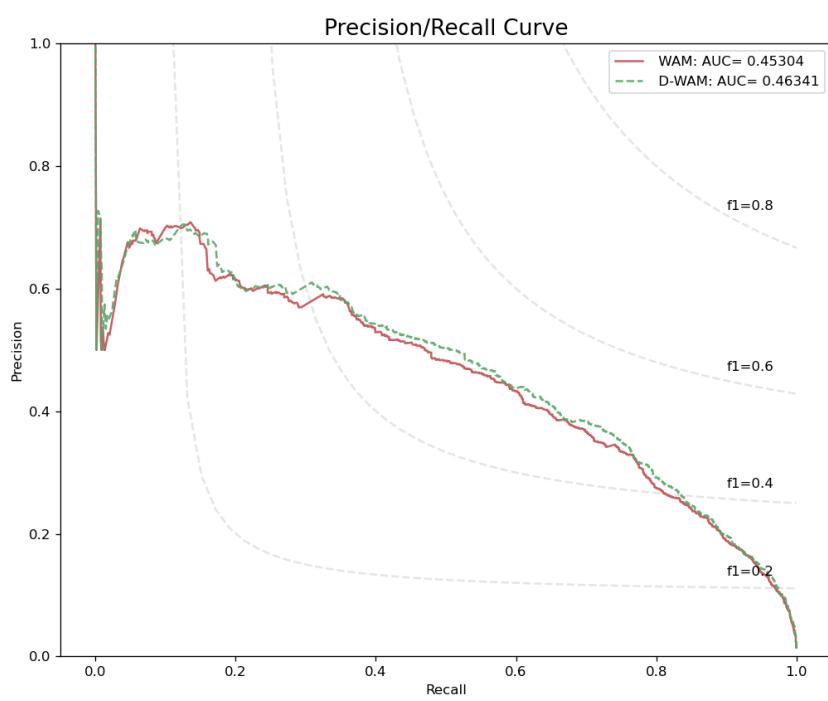
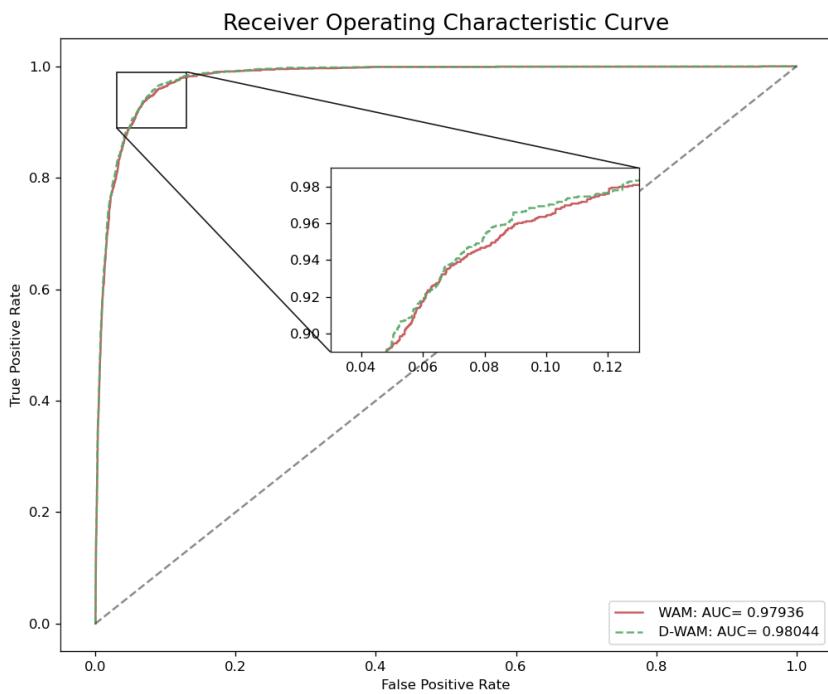


图 13. Double-WAM的ROC和PR图

5 DISCUSSION & CONCLUSION

5.1 模型性能

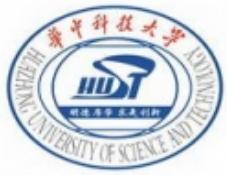
本报告通过调整 donor 位点上下游碱基序列长度，比较不同滑动窗口的预测性能，选取最佳的 signal 序列长度为 [-4,6]；WAM 考虑了双碱基之间的关联，预测结果比 WMM 更好，但总体来说效果还是差强人意，Precision 最高只将近 0.7，也就是说无论如何调整阈值，预测为 donor site 的正样本中都含有很大一部分假样本，假阳性较高。最后，我还尝试使用双层 WAM 对模型进行改进，但预测结果也只有些许提升。

5.2 关于改进

- WAM 虽然考虑了碱基间的关联，但只考虑了相邻碱基的关联，但没有探索三个以上碱基的相互关系。可以进一步尝试更多碱基间的相互关系；
- 训练集会较大程度影响到模型性能。可以寻找其他公开的基因数据集，查看 WAM 在不同模型的预测效果；
- 从碱基序列只能获得新的生物学信息，可以利用其他生物学信息，例如利用 pre-mRNA 的结构信息来帮助预测剪接位点；
- 目前只用 WAM 预测了 donor site，可以尝试预测 acceptor site 比较两者差异。

6 REFERENCE

1. Steven L. Salzberg, A method for identifying splice sites and translational start sites in eukaryotic mRNA, *Bioinformatics*, Volume 13, Issue 4, August 1997, Pages 365–376, <https://doi.org/10.1093/bioinformatics/13.4.365>
2. M.O. Zhang, T.G. Marr, A weight array method for splicing signal analysis, *Bioinformatics*, Volume 9, Issue 5, October 1993, Pages 499–509, <https://doi.org/10.1093/bioinformatics/9.5.499>
3. Baten, A., Chang, B., Halgamuge, S. *et al.* Splice site identification using probabilistic parameters and SVM classification. *BMC Bioinformatics* 7, S15 (2006). <https://doi.org/10.1186/1471-2105-7-S5-S15>



华中科技大学

生物信息数据挖掘报告

Prediction of Splicing Sites by SVM

授课教师 : 周艳红
课程名字 : 生物信息数据挖掘
班 级 : 生信基地1801
姓 名 : 苏济雄
学 号 : U201812416
日 期 : 2021.06.12

Prediction of Splicing Sites by SVM

Author: Jixiong Su

Huazhong University of Science and Technology

Abstract: In this paper, I use Support Vector Machine to predict donor site in eukaryotic genes, with One-Hot Encoding and different lengths of samples to explore different kernels of SVM. Finally the result is that SVM has the best predictive ability for sequences of 20 upstream sites and 20 downstream sites from exon/intron boundary.

Key Words: SVM, Machine Learning, Gene Finding, Splice Site.

1 AIM

- Fully understand the mathematical principle of Support Vector Machine(SVM), kernel function type and kernel parameter selection principle.
- Use SVM to predict donor sites, using linear, polynomial and gaussian kernels respectively.

2 BACKGROUND

Among the various tools in computational genetic research, gene prediction remains one of the most prominent tasks. Accurate gene prediction is of prime importance for the creation and improvement of annotations of recently sequenced genomes . In the light of new data related to natural variation, the importance of accurate computational gene finding gains increasing importance since it helps to understand the effects of polymorphisms on the gene products.

In eukaryotic genes, splice sites mark the boundaries between exons and introns. The latter are excised from premature mRNAs in a post-processing step after transcription. Both the donor sites at the exon-intron junctions, and the acceptor sites at the intron-exon boundaries, have quite strong consensus sequences which can, however, vary significantly from one organism to another. The vast majority of all splice sites are so called canonical splice sites which are characterised by the presence of the dimers GT and AG for donor and acceptor sites, respectively. The occurrence of the dimer is not sufficient for the splice site. Indeed, it occurs very frequently at non splice site positions.

We therefore face two extremely unbalanced classification tasks, namely the discrimination between true donor sites and decoy positions with the consensus dimer GT and the discrimination between true acceptor sites and decoy positions with the consensus dimer AG.

3 METHODS:

3.1 Description of models

Support vector machines (SVMs) are a particularly powerful and flexible class of supervised algorithms for both classification and regression.

The advantages of support vector machines are:

- Effective in high dimensional spaces.
- Still effective in cases where number of dimensions is greater than the number of samples.
- Uses a subset of training points in the decision function (called support vectors), so it is also memory efficient.
- Versatile: different Kernel functions can be specified for the decision function. Common kernels are provided, but it is also possible to specify custom kernels.

The disadvantages of support vector machines include:

- If the number of features is much greater than the number of samples, avoid over-fitting in choosing Kernel functions and regularization term is crucial.
- SVMs do not directly provide probability estimates, these are calculated using an expensive five-fold cross-validation.
- The scaling with the number of samples N is $O[N^3]$ at worst, or $O[N^2]$ for efficient implementations. For large numbers of training samples, this computational cost can be prohibitive.
- The results are strongly dependent on a suitable choice for the softening parameter C . This must be carefully chosen via cross-validation, which can be expensive as datasets grow in size.

3.2 Algorithms

Fomula:

$$\begin{aligned} & \min_{\mathbf{w}, b, \xi \geq 0} \frac{1}{2} \mathbf{w}^\top \mathbf{w} + C \sum_i \xi_i \\ & \text{s.t. } y_i (\mathbf{w}^\top \mathbf{x}_i + b) \geq 1 - \xi_i, \quad i = 1, \dots, n \\ & \quad \xi_i \geq 0 \end{aligned}$$

Kernel Function:

Kernel Function is a method used to take data as input and transform into the required form of processing data. “Kernel” is used due to set of mathematical functions used in Support Vector Machine provides the window to manipulate the data. So, Kernel Function generally transforms the training set of data so that a non-linear decision surface is able to be transformed to a linear equation in a higher number of dimension spaces. Basically, It returns the inner product between two points in a standard feature dimension.

1. Linear Kernel :

$$\text{kernel} = \langle x, x' \rangle$$

It is mainly used in the case of linear separability. For general data, the classification effect is very ideal.

2. Gaussian Kernel Radial Basis Function (RBF):

$$\text{kernel} = \exp(-\gamma \|x - x'\|^2)$$

Radial basis function = Gaussian kernel.

It is mainly used in the case of linear indivisibility, and the classification results are very parameter dependent.

3. Poly Kernel :

$$\text{kernel} = (\gamma \langle x, x' \rangle + r)^d$$

It represents the similarity of vectors in training set of data in a feature space over polynomials of the original variables used in kernel.

Parameter:

- degree is the degree of the polynomial.
- gamma is the coefficients of polynomial.
- coef0 stands for r, offset the function from zero.

4. sigmoid kernel:

this function is equivalent to a two-layer, perceptron model of neural network, which is used as activation function for artificial neurons.

$$\text{kernel} = \tanh(\gamma \langle x, x' \rangle + r)$$

3.3 Program design

3.3.1 feature extraction and data coding

Data processing method:

- Extraction of donor site length: in order to evaluate the impact of sample length on prediction, different lengths of singal sequences are selected to view different prediction results.
- Base encoding: adopts one-hot coding method, set up A code for [1,0,0, 0], G for [0,1,0,0], C [0,0,1,0], T [0,0,0,1], Z (unknown base) for [0,0,0,0].
- Set negative sample: the negative sample is set to a sequence containing GT base pair but not real donor site.
- Label: Set label to 1 for positive samples and 0 for negative samples.

Data extraction results:

- Each file in the training set and test set represents an eukaryotic gene. There is at least one exon and intron in each sequence, and all sites conform to the AG-GT rule, that is, all donor sites are GT, and all acceptor sites are AG. Finally, 2381 true donor signal sequences are extracted from 462 training dataset.
- 2079 true donor signal sequences and 149255 pseudo signals are extracted from 570 files in the test dataset.

3.3.2 kernel function selection

The kernel functions of SVM are RBF, linear and poly. The training speed of linear is the fastest, and the effect is good in the case of linear separability. The training speed of RBF is the slowest, but it can fit better in the case of nonlinear, but it depends on parameters very much. The poly kernel is in the middle. This experiment will compare the performance of three kernel functions in the prediction of donor site.

3.3.3 Parameter learning

Superparameters are parameters that cannot be directly learned by the model, which need to be set manually, or be found through superparameter optimization algorithms such as Bayesian optimization or Grid Search. For example, the superparameters of SVM include regularization parameter C, kernel coefficient containing gamma, degree, class weight, cofe0, etc. When adjusting the superparameters, it might have been overfit on the test dataset, need to have a validation set so that training on the training set and evaluating on the validation set, and if it works well, you can manage the final evaluation on the test dataset. Note that when adjusting parameters, we can't use test dataset for verification, because our purpose is to apply training model to unseen data.

I finally used k-folded cross-validation to evaluate the training effect of the model. In the k-folded cross-validation, the data we used are all the data in the training set .Randomly divide a dataset into k groups, or “folds”, of roughly equal size. Choose one of the folds to be the holdout set. Fit the model on the remaining k-1 folds. Calculate the validation scores on the observations in the fold that was held out. Repeat this process k times, using a different set each time as the holdout set. Calculate the overall validation scores to be the average of the scores.

The function GridSearchCV is used to find the best parameters and perform 3x cross-validation simultaneously.

- C: it can adjust the extreme value of the penalty, control the size of the gap between the two lines, on behalf of the soft gap. When C is too large, the division is more strict, the generalization ability is weaker, and it is easy to over fit.
- Gamma:
 - The larger the gamma value, the higher the dimension of the mapping and the more complex the model, which may make all points become support vectors.
 - The smaller the gamma, the simpler the model.

3.3.4 prediction and performance evaluation

Choosing the best parameters, I use three kernel functions to predict whether the sample contains donor site, and compare the performance of the three models and the difference between SVM and WAM by ROC and PR plot.

ROC curve is a curve reflecting the relationship between sensitivity and specificity. The closer the ROC curve is to the upper left corner, the higher the accuracy of the test. The point closest to the top left corner of the ROC curve is the best threshold with the least errors, and the total number of false positives and false negatives is the least. AUC is the area under the ROC curve. The meaning of AUC probability is to randomly take a pair of positive and negative samples, and the probability that the score of positive samples is greater than that of negative samples. AUC is robust to the unbalanced distribution of positive and negative samples, and it is a very common measure of classifier.

Precision-Recall is a useful measure of success of prediction when the classes are very imbalanced(PR plot for short). In information retrieval, precision is a measure of result relevancy, while recall is a measure of how many truly relevant results are returned. When the distribution of positive and negative samples is unbalanced, the ROC curve remains unchanged, while the PR curve changes greatly. Compared with the ROC plot, the PR plot can reflect the ability of the model to identify positive samples.

4 RESULTS

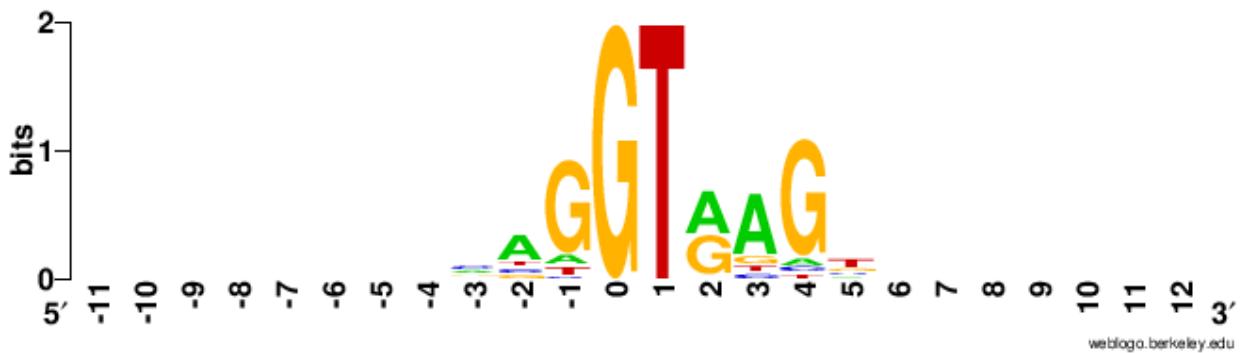


Fig 1. The sequence logo.

Figure 1 shows the sequence logo of 11 upstream bases and 11 downstream bases of all donor site GT base pairs in the training dataset. A sequence logo is a graphical representation of an amino acid or nucleic acid multiple sequence alignment. Each logo consists of stacks of symbols, one stack for each position in the sequence. The overall height of the stack indicates the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each amino or nucleic acid at that position. In general, a sequence logo provides a richer and more precise description of, for example, a binding site, than would a consensus sequence.

From the sequence logo of the upstream 11 bases and the downstream 11 bases from the GT

base pair , we can find that the bases at sites -3 to 5 are conservative, while the base frequencies at other sites are disordered, so I choose -3 to 5 as the signal sequence. However, considering that SVM may be able to find unknown base associations, I also choose other lengths to explore the model effect, using SVM function in the Python Sklearn package for training and predicting.

4.1 Window=[-3, 6]

Select a window of 9 base positions was chosen for the donor site, where 3 consecutive bases are upstream from the exon/intron boundary and 6 consecutive bases are downstream to the exon/intron boundary. A total of 283780 pseudo signal were extracted from 9 base length samples. Due to the large amount of data, 15916 pseudo signals are extracted after removing the duplication finally.

Table 1. The Paramters of Different Kernels for Sample Length = 9

Kernel	Parameters
RBF	C=0.1,gamma=1,class_weight='balanced'
Linear	C=0.1,class_weight='balanced'
Poly	C=0.1,degree=4,gamma=1,class_weight='balanced'

Table 2. The Default Evaluation Results for Sample Length = 9

model	precision	recall	f1-score
rbf	0.224919	0.896104	0.359583
poly	0.190185	0.928331	0.315695
linear	0.115289	0.96152	0.205891

Table 1 shows the paramter selected. Table 2 shows the default evaluation results for the three models. It can be seen that the F1-score of RBF nucleus is the highest, while the recall value of linear nucleus is the highest

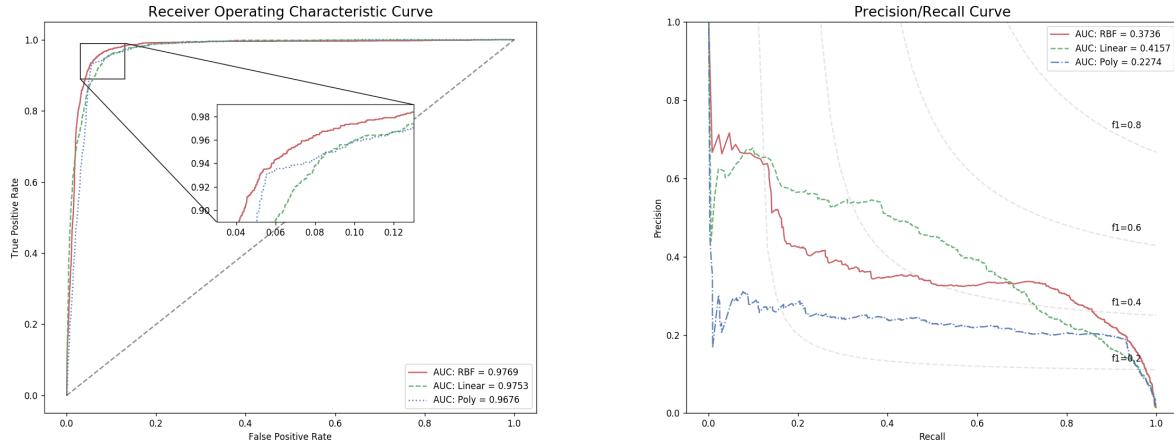


Fig 2. ROC plot and PR plot where 3 consecutive bases are upstream from the exon/intron boundary and 4 consecutive bases are downstream to the exon/intron boundary

From the ROC, it can be seen that the AUC of RBF is the highest up to 0.9769, which is quite good, but there is not much difference between RBF and Linear, and the predicting effect of Poly Kenrel is not good (maybe the parameters are not set well).

According to the PR curve, the AUC of linear kernel is the highest, and when Recall is 0.1-0.7, Precision is higher than that of other kernels, but when Recall is 0.7-1.0, Precision of RBF is highest.

I select RBF to make the tendency of Recall and Precision under different thresholds. It can be seen that F1-score keeps rising. When the threshold is set to 0.9, the F1-score is 0.457, and the Recall is 0.730 while the Precision is 0.333, not bad.

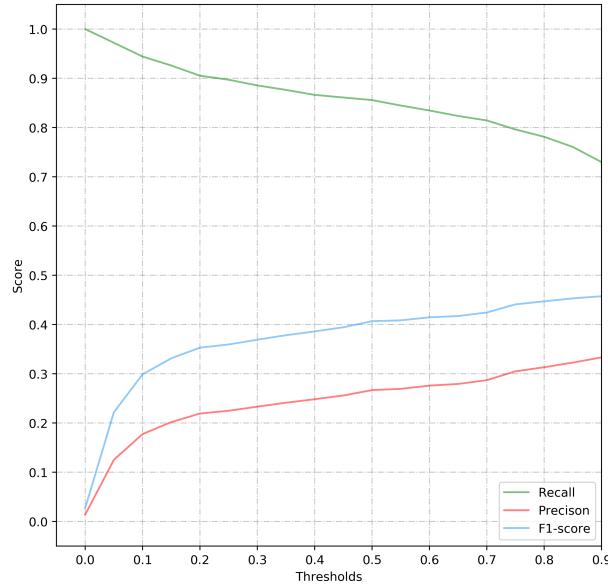


Figure 3 The Tendency of Recall and Precision under different thresholds in RBF kernel when sample length = 9.

4.2 Window=[-3, 10]

Although it can be seen from the above weblogo that the information entropy after the fifth site upstream from GT is very small, I still hope that SVM can find the hidden information, so I try selecting a window of 13 base positions where 3 consecutive bases are upstream from the exon/intron boundary and 10 consecutive bases are downstream to the exon/intron boundary.

A total of 283712 pseudo signals are extracted, which have little effect after duplication, so $1 / 10 = 28371$ samples are randomly extracted.

Table 3. The Parameters of Different Kernels for Sample Length = 13

Kernel	Parameters
RBF	C=1, gamma=1, class_weight='balanced'
Linear	C=1, class_weight='balanced'
Poly	C=1, degree=4, gamma='scale', class_weight='balanced'

Table 4. The Default Evaluation Results for Sample Length = 13

model	precision	recall	f1-score
rbf	0.709534	0.46176	0.559441
poly	0.233561	0.919192	0.372478
linear	0.128707	0.9519	0.226755

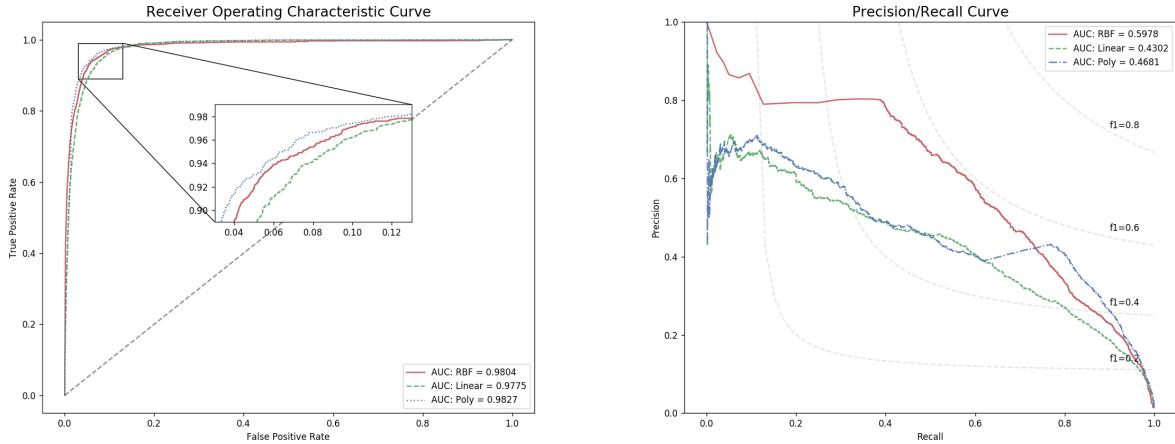


Fig 4. ROC plot and PR plot where 3 consecutive bases are upstream from the exon/intron boundary and 10 consecutive bases are downstream to the exon/intron boundary

Surprisingly, the result is better than that of the previous 9 base samples. The AUC values of the three nucleuses are all higher than those of the previous one. On the contrary, the Poly nucleus exceeded the linear Kernel and the RBF Kernel, and the AUC reach 0.9827.

As can be seen from PRC ,the curves are also significantly improved, and the AUC of RBF reaches 0.5978. When Recall is from 0 to 0.7, Precision in RBF Kernel is significantly higher than that of the other two kernels, while Recall of the Poly kernel is from 0.8 to 1.0 , still maintains a good precision.

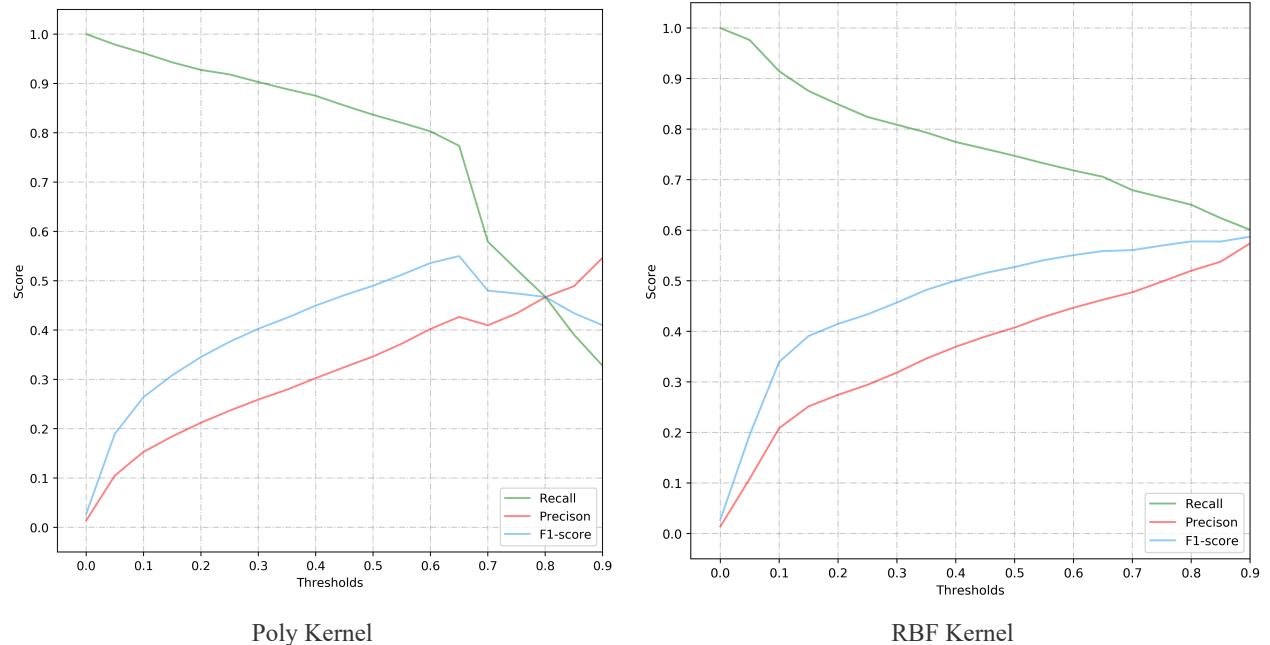


Fig 5. The Tendency of Recall and Precision under different thresholds in Poly Kernel and RBF kernel when sample length = 13.

4.3 Window=[-20, 20]

Select a window of 40 base positions was chosen for the donor site, where 20 consecutive bases are upstream from the exon/intron boundary and 20 consecutive bases are downstream to the exon/intron boundary for training.

Table 5. The Parameters of Different Kernels for Sample Length = 40

Kernel	Parameters
RBF	C=0.1, class_weight={0: 1, 1: 100}, gamma=1, kernel: 'rbf'
Linear	C=1, class_weight='balanced'
Poly	C=1, degree=4, gamma='scale', class_weight='balanced'

Table 6. The Default Evaluation Results for Sample Length = 40

model	precision	recall	f1-score
rbf	0.300529	0.929774	0.454236
poly	0.49357	0.830688	0.619218
linear	0.143736	0.955748	0.24989

The results are surprisingly good. The AUROC of Poly kernel is up to 0.9903, the RBF kernel and the linear kernel are up to 0.9896 and 0.9804 respectively. The AUPRC of Poly kernel is up to 0.7007, while the RBF kernel is up to 0.6665.

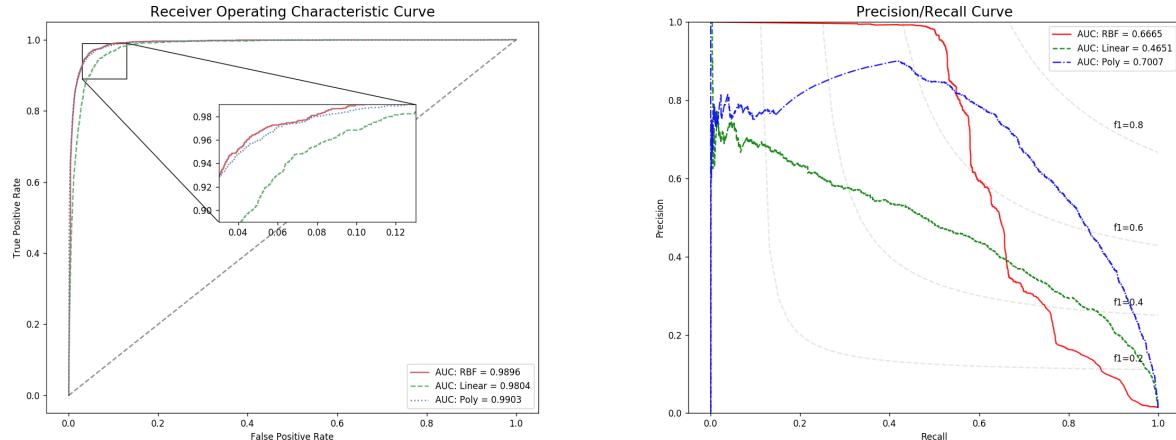


Fig 6. ROC plot and PR plot where 20 consecutive bases are upstream from the exon/intron boundary and 20 consecutive bases are downstream to the exon/intron boundary

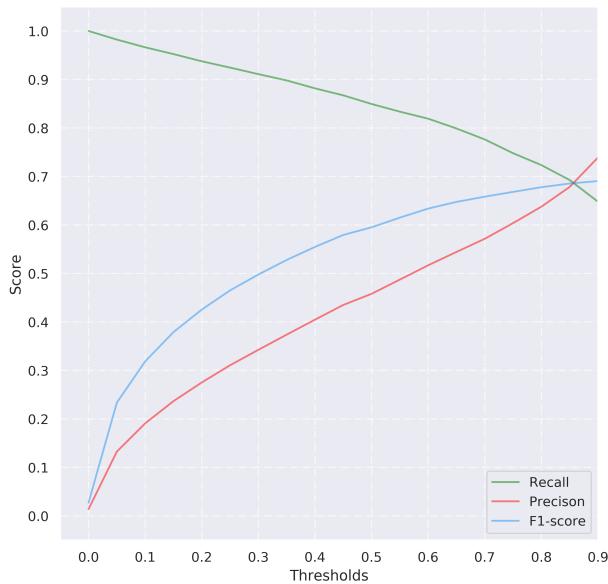


Fig 7. The Tendency of Recall and Precision under different thresholds in Poly Kernel when sample length = 40.

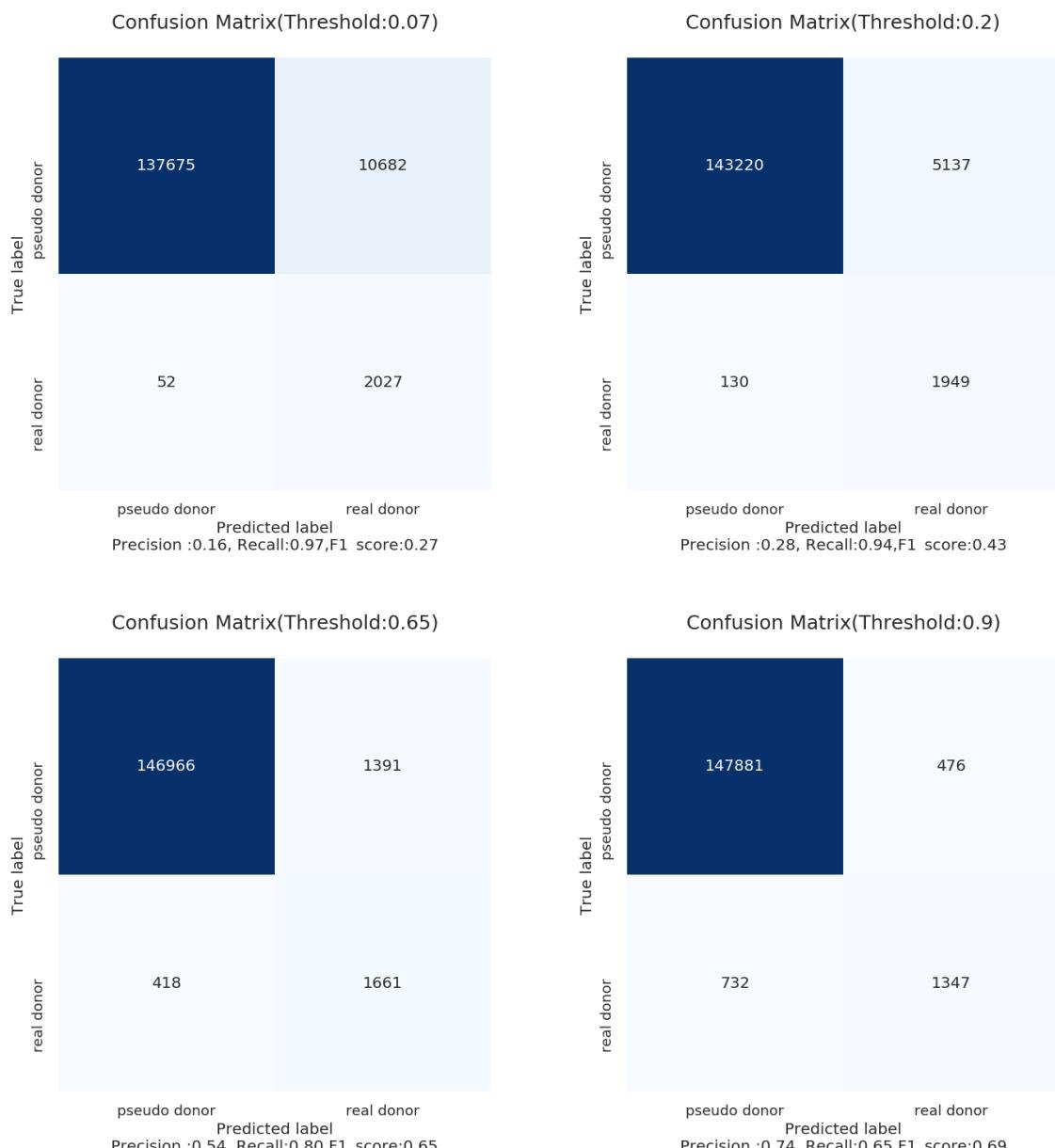


Fig 8. Confusion Matrixes under different thresholds in Poly Kernel

5 DISCUSSION & CONCLUSION

In this experiment, I selected signal sequences of three kinds of lengths as samples for training, and used One-hot Coding. And I learned that SVM has the best predictive ability for sequences of 20 upstream sites and 20 downstream sites of exon/intron boundary, which hidden connections near donor site may be distinguished by SVM.

Overall predicting performance: Polynomial kernel \approx RBF kernel $>$ Linear kernel . Note that Polynomial kernel and RBF kernel are very parameter-dependent, so the result I get maybe not the best result.

Improvement:

- For the sample length, only three schemes are selected, and more schemes can be tried. Maybe the prediction effect of the model will be better
- Due to the high time complexity of SVM, there is not much time to search the optimal parameters, which may stimulate the real potential of the model
- Due to the excessive number of training samples, we can only adopt the method of de-duplication or random sampling, which may cause some loss of information of training samples. It may be necessary to find a suitable solution.
- Only using the BG set as testing set to view the model predictive ability, need to use more data sets to prove the model's generalization ability

6 REFERENCE

1. ML-NLP/4. SVM.md at master · NLP-LOVE/ML-NLP (github.com)
2. 05.07-Support-Vector-Machines.ipynb - Colaboratory (google.com)
3. 【python 机器学习笔记】SVM 实例：有毒蘑菇预测 - 知乎 (zhihu.com)
4. 四、支持向量机（SVM） - 知乎 (zhihu.com)
5. sklearn 之 SVM，ROC 曲线与 AUC 面积_WANGBINLONG-的博客-CSDN 博客
6. Python 中的支持向量机 SVM 的使用（有实例） - 陆瑶 - 博客园 (cnblogs.com)
7. Platt J. Probabilistic outputs for support vector machines and comparisons to regularized likelihood methods[J]. Advances in large margin classifiers, 1999, 10(3): 61-74.
8. Cost-Sensitive SVM for Imbalanced Classification (machinelearningmastery.com)
9. SVM（支持向量机）——原理 - 知乎 (zhihu.com)
10. 机器学习篇-指标：AUC - 知乎 (zhihu.com)

