LncPTPred: Predicting lncRNA-Protein Interaction based on Crosslinking and Immunoprecipitation (CLIP-Seq) Data

Gourab Das[1], Troyee Das[1] and Zhumur Ghosh[1*]

[1]Department of Biological Sciences, Bose Institute, Unified Academic Campus, Kolkata-700 091, India

[*] To whom correspondence should be addressed. Tel:+91-0332569 3220; Email: zhumur@jcbose.ac.in

ABSTRACT

Long non-coding RNA (lncRNA)-Protein Interaction (LPI) across diverse biological systems directly and indirectly regulates various cellular processes. Experimental assays to recognize the protein binding partners of lncRNAs is highly time consuming and expensive. *In-silico* predictive approaches involving pattern recognition technique provides a promising alternative to it by reducing the search space. Our work identifies such hidden pattern from within the Cross-linking immunoprecipitation sequencing (CLIP-Seq) data which aids to overcome the problem of obtaining real negative dataset and thus offers a state-of-the-art machine learning (ML) based prediction algorithm to predict LPI. Initial phase of this work involves preparation of the training dataset and the next phase is devoted towards developing the ML based model to perform prediction operation. In order to show the efficacy of our model, its performance has been compared against that of the contemporary prediction tools, where the result clearly shows the outperformance of our model. Moreover it also provides the segments of interaction within the lncRNA loci which acts as a roadmap for precise designing of the validation experiment. The LncPTPred tool has been provided in terms of web server as well as standalone version in github**.**

Web server Link: http://bicresources.jcbose.ac.in/zhumur/lncptpred/

Github Link: https://github.com/zglabDIB/lncptpred.git

Keywords: lncRNA-Protein Interaction, CLIP-Seq, Machine Learning, Hyperparameter Optimization

INTRODUCTION

Long noncoding RNAs (lncRNAs) play vital role towards regulating a wide spectrum of biological processes including gene transcription, epigenetic modifications along with other post-transcriptional regulatory mechanisms. These versatile molecules are poorly conserved and adopt various mechanisms to implement such regulatory actions which includes RNA-RNA(1), RNA-protein (2) and competing endogenous RNA (ceRNA) (3) mode of interaction. Among these, lncRNA-protein interaction(LPI) constitutes one such important mode of interaction which not only have implication in various normal biological processes and different disease systems but also influence lncRNA processing, their modifications, stability and function (4). The advancement of next gen sequencing techniques have facilitated the detection of RNA-protein interaction viz. cross-linking immunoprecipitation-seq (CLIP-seq) (5) and RNA immunoprecipitation seq (RIP-seq) (6), RIP-Chip etc. However, irrespective of the nature of

the experiments, they are costly and time-consuming. Alternatively, *in-silico* approaches like Molecular docking(MD) and ML based approach have been adopted for quick screening of potential RNA-protein interaction, more specifically LPIs. But MD being computationally expensive have necessitated the introduction of Artificial Intelligence (AI) based computational tool to predict the LPI binding interactions. Thus Machine learning (ML) or Deep learning(DL) based approaches have gained importance which provides a time optimized solution.

There have been reports on ML based RPI prediction models incorporating either sequence based or structure based features. Initial study by Pancaldi et al. (7) mentioned mRNA-protein target prediction using Random Forest (RF) and Support Vector Machine (SVM) models. They have incorporated various features associated with the genes and proteins of the *Saccharomyces cerevisiae* (yeast). Muppirala et al. (8) created RPISeq using same RF and SVM models with 3-mer protein and 4-mer RNA features from the sequences. Wang et al. (9) trained Naïve-Bayes classifier for RPI and Lu et al. (10) proposed lncPro to generate association score using Fisher linear discriminant analysis (11). RPI-Pred (12) model made use of both sequence and structural features to train SVM classifiers whereas IPMiner (13) used deep learning based stacked autoencoder to create computational RPIs. Some of the recent studies related to LPI prediction includes Human lncRNA-protein interactions (HLPI)-Ensemble (14) based ensemble models in terms of RF, Extreme Gradient Boosting (XGBoost) and SVM based general model. Li et al. (15) proposed Capsule-LPI tool incorporating multimodal features in terms of secondary structure, motif, sequence and physiochemical properties for both lncRNA and protein where Capsule network forms the basis of their prediction model. LPI-CNNCP (16) used Convolution Neural Network (CNN) model to perform prediction task where the feature set was generated using copy padding trick to convert variable length sequences of lncRNA/protein into fixed length inputs, incorporating one-hot encoding in order to transform CNN-compatible image data. LPI-HyADBS (17) formed hybrid network containing deep neural network (DNN), XGBoost, and SVM with a penalty Coefficient of misclassification (C-SVM) to execute prediction strategy by incorporating Pyfeat (18) based biological features, extracted from lncRNA and proteins sequence. EnANNDeep (19) constructs ensemble framework containing adaptive k-nearest neighbor classifier, deep neural network, and deep forest to perform prediction task where features from multiple sources are integrated to determine lncRNA-protein pair. The most predominant datasets used as training data by these specified models are NPInter (20), PRIDB (21) & RPIs (8,13) which contain only real positive interaction pairs**.**

It is important to emphasize that the biggest pitfalls corresponding to most of these models is the non-availability of real non-LPI dataset or real negative dataset. Negative dataset were generated by random shuffling of unlabelled interactions. Thus, it induces noise within trained models that leads to inaccurate learning. In this work, we have developed LncPTPred; an LPI prediction tool to address this issue by generating properly labeled dataset yielded from CLIP-Seq based biological assays consisting of both Photoactivatable Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation (**PAR-CLIP**) (22) and High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (**HITS-CLIP**) (23). Guiding principle corresponding to our training data generation procedure considers that RNA binding Protein found from PAR-CLIP and HITS-CLIP sequences are positive data and rest of the non-interacting segments are the negative one. Moreover, it is able to predict the exact location of interaction which is not provided by any other state-of-the-art prediction tools mentioned above. Further, the performance of LncPTPred has been compared against other state-of-the-art LPI prediction models. Finally, we have tested our models deployed for LncPTPred by correctly predicting well known LPIs in case of certain diseases. User can access LncPTPred from http://bicresources.jcbose.ac.in/zhumur/lncptpred/ . It's standalone version is available at https://github.com/zglabDIB/lncptpred.git .

MATERIAL AND METHODS

The detailed workflow associated with LncPTPred depicted in **Figure 1** can be segregated into two main modules. **Initial module deals** with **Data Curation** where LPI data has been extracted from PAR-CLIP and HITS-CLIP based CLIP-Seq assay. Numerous preprocessing operations have been incorporated in order to filter out lncRNA sequence segments from the list of all binding sequences. **The second module** is dedicated towards developing Feature Creations and **Machine Learning Models** where two of the main sub-modules include development of stacking classifier based Meta learner using **scikit-learn** (https://scikit-learn.org/stable/) (24) and **Hyperparameter Optimization** using **Optuna** (25). Finally there is the Output Module and data visualization part which provides the details regarding the interacting lncRNA segment. Information on each module as well as curated datasets is detailed below.

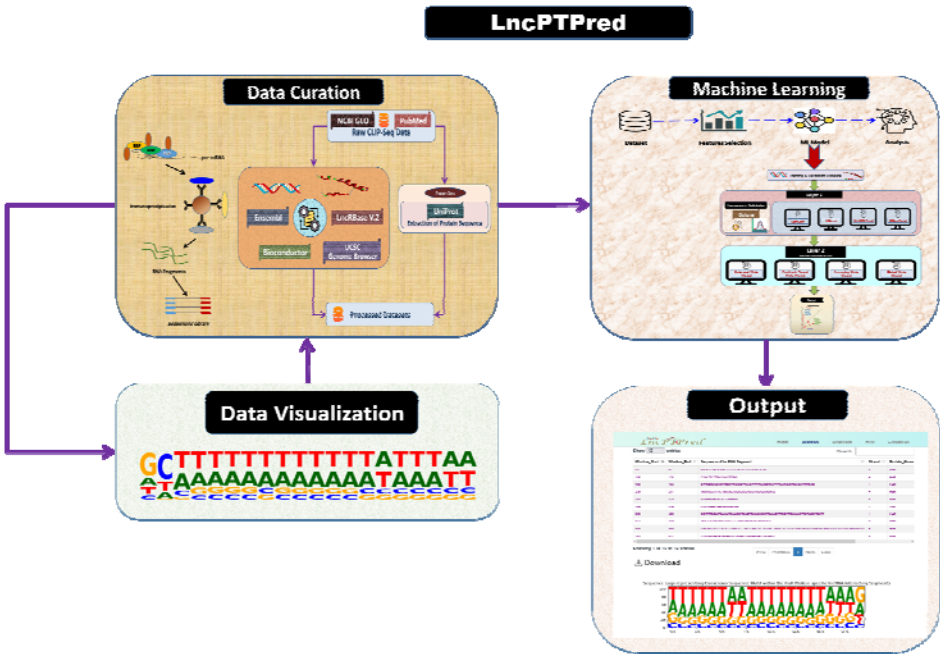


Figure 1: Workflow of the entire work

The flow diagram depicts the various steps involved towards development of LncPTPred which includes Data Curation from CLIP-Seq data, development of the ML based models, output generation and data visualization

Dataset:

Given the maximal importance towards the generation of properly labelled dataset we have considered CLIP-Seq experimental datasets viz. PAR-CLIP (22) and HITS-CLIP (23) data. For a particular protein, protein bound RNA fragments have been considered as positive data and rest of the non-bounded fragments have been considered as real negative data. These data have been curated from GEO (https://www.ncbi.nlm.nih.gov/geo/) and pubmed ID: 22081015, as provided in **Supplementary File** 1 in detail. The entire set of GSE numbers, the pubmed ID of the input datasets constituting the training and validation data along with the protein names and cell line information is provided in **Table 1**.

GSE_NUMBER/Pubmed_Id	Protein_Name	Cell_Line
GSE38201	ALKBH5	HEK293
GSE98856	G3BP1	HeLa
GSE160948	SF3B1	MDA-231-LM2
GSE46908	LIN28B	HEK293
GSE38201	C17orf85	HEK293
GSE102320	HuR	293T
GSE111900	SRSF2	Human erythroid leukemia (HEL) cell line
GSE130867	SRSF1	H1299
GSE133724	FMR1	HEK293
GSE160949	SNRPA1	MDA-231-LM2
GSE98836	EWSR1	HeLa
GSE173628	ESR1	MCF7
GSE38201	CAPRIN1	HEK293
GSE38201	C22orf28	HEK293
GSE62115	MSI2	K562
GSE44615	LIN28A	HEK293
GSE44615	LIN28B	HEK293
GSE47078	RC3H1	HEK293
GSE58352	YTHDC1	HeLa
GSE111188	QKI	Mesenchymal-like HMLE cells
GSE39872	LIN28A	H9
GSE38201	ZC3H7B	HEK293
GSE63591	YTHDF1	HeLa
GSE49339	YTHDF2	HeLa
GSE19323	PTBP2	HeLa
GSE52977	AUF1	HEK293
GSE46705	METTL14	HeLa
GSE29943	HuR	HeLa
GSE46705	METTL3	HeLa
GSE46705	WTAP	HeLa
GSE150925	YBX1	HEK293
GSE102336	ZCCHC4	Hela
GSE53184	ZFP36	HEK293
Pubmed:22081015	TAF15	HEK293
Pubmed:22081015	FUS	HEK293

Table 1: *Input Training Dataset utilized for developing LncPTPred*

The unknown test data have been provided in **Table 2**. Both the dataset in Table 1 and Table 2 undergoes several pre-processing steps as done under the 1st Module named Data curation.

GSE_NUMBER/Pubmed_Id	Protein_Name	Cell_Line
GSE98856	G3BP2	HeLa
Pubmed:22081015	EWSR1	HEK293

Table 2: *Unknown Test Dataset utilized to validate the performance of LncPTPred against external models*

Data Curation:

Datasets of various CLIP-Seq experiments mined from GEO database were in diverse formats such as excel, text, bed, bedgraph and bigwig. Moreover, different build version of human sequences like **hg38, hg19** or even **hg18** have been considered while generating the CLIP-seq data as detailed in **Supplementary File 1**. It necessitates the incorporation of robust pre-processing techniques in order to extract the protein bound RNA segments corresponding to a particular CLIP experiment. Furthermore, it is essential to screen out lncRNAs from the given set of protein bound fragments. The flowchart in **Figure 2** depicts the different steps(as detailed below) to generate properly labeled data to be utilized by ML based target prediction model.

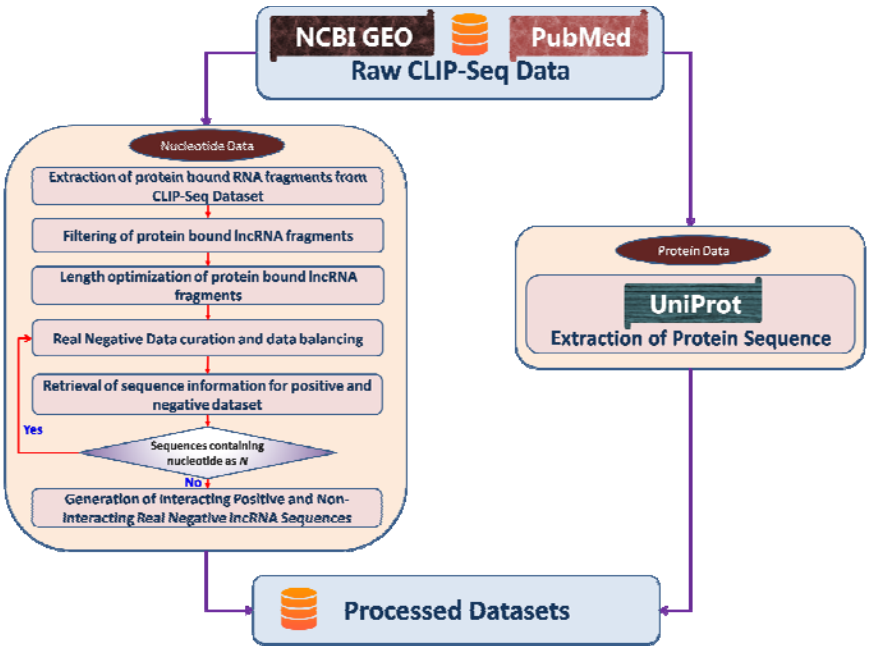


Figure 2: Data Curation Workflow

It depicts the pre-processing of the raw CLIP-seq data to build the training dataset, validation

and unknown test dataset which includes the extraction of positive and real negative data. It also depicts the curation of protein sequence information

(a) Extraction of protein bound RNA fragments from CLIP-Seq Dataset:
Protein bound RNA fragments have been extracted from the downloaded files in various formats viz. bed, bedgraph, excel, text and bigwig. More specifically the start & end location, strand and chromosome specific information of these protein bound RNA fragments have been obtained.

(b) Filtering of protein bound lncRNA fragments:
The reference files were created at first, prior to mapping the protein bound RNA fragments with them. This has been done using Ensembl Biomart (26) and LncRBase V.2 (27) as the reference database corresponding to human genome version **hg38**.
Since some of the input dataset have been generated using genome build hg19 and hg18, UCSC genome liftover tool (http://genome-euro.ucsc.edu/cgi-bin/hgLiftOver) have been used to convert **hg38** reference file into **hg19 and** CrossMap (28) tool (https://genviz.org/module-01-intro/0001/06/02/liftoverTools/) to convert hg19 reference file to hg18. The reference files are further filtered with length cut off of <200 bases as lncRNA length is typically ≥ 200 bases. All the reference files contain chromosome number, strand and start & end position of the lncRNA loci.

Subsequently, the protein bound RNA fragments have been mapped to these reference files to filter out the protein bound lncRNA fragments. The pseudocode for performing the mapping at this step is as follows:
Input: *All RNA fragments of given CLIP-Seq experiments & list of all lncRNAs from Reference file.*
Output: *list of lncRNA fragments from given CLIP-Seq experiments*

```
INITIALIZE clip_data:=table containing all RNA fragments of given experiments
INITIALIZE ref_data:= table containing all lncRNA from Reference

FOR each chromosome
  ASSIGN clip_sub_data:= subsets from clip_data of given chromosome
  ASSIGN ref_sub_data:= subsets from ref_data of given chromosome

    FOR each RNA fragments R_i of clip_sub_data
      ASSIGN clip_sub_interval:= interval containing start and end location of given RNA R_i

      FOR each lncRNA of ref_sub_data
        ASSIGN ref_sub_interval:= interval containing start and end location of each lncRNA

       IF (clip_sub_interval overlaps ref_sub_interval)
         ACCEPT R_i
       ELSE
         REJECT R_i
       ENDIF

      ENDFOR
```

ENDFOR
ENDFOR

(c) Length optimization of protein bound lncRNA fragments:
We next checked the distribution of the lengths of these protein bound lncRNA fragments in training, validation and unknown test dataset. Violin plot provided in **Figure 3** shows that the length of the protein bound lncRNA fragment size dominating around a threshold of ≤ 40 bases which is well supported by earlier reports (29). This is an important parameter for determining the window size of the search space to be selected by the users while using LncPTPred. In order to remove length bias without ignoring too much data segments we have filtered the length of lncRNA between 10 and 200 nucleotides.

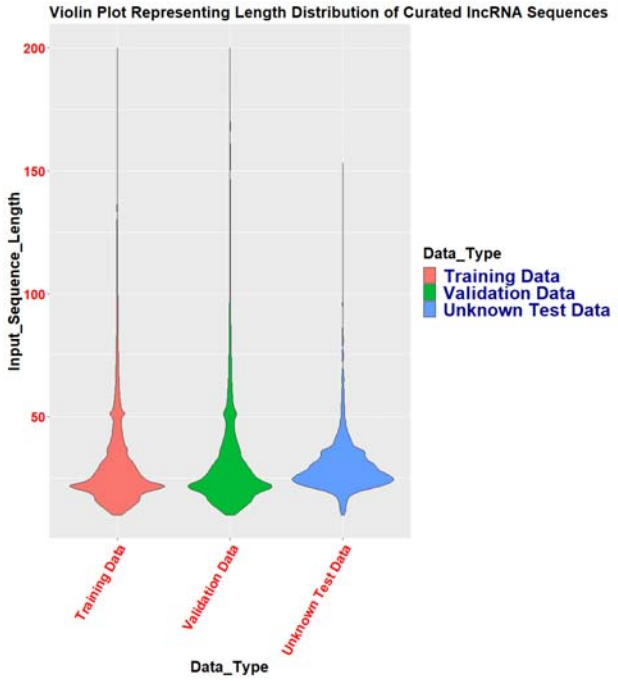


Figure 3: Length Distribution of Interacting lncRNA sequences extracted from CLIP-seq data

The violin plot shows the length distribution of interacting lncRNA sequences extracted from CLIP-seq data subdivided as training, validation and unknown test dataset which will serve as an important parameter for determining the window size of the search space to be selected by the users while using LncPTPred.

(d) Real Negative Data curation and data balancing:
In a CLIP-Seq experiment for a particular protein, RNA fragments not bound to the protein have been considered as real negative data. In order to address data imbalance due to more number of negative data as compared to positive data, random sampling of non-bound lncRNA fragments (for a particular protein) have been done so as to maintain consistency with the positive dataset in terms of number, length and strands of the fragmented sequences. Corresponding to each positive dataset, we have generated negative data belonging to same chromosome and strands across 10KB to 1 MB cis-location on either side of the given positive data. The detailed pseudocode for negative data curation is given below:

Input: *All binding RNA and lncRNA fragments of given CLIP-Seq experiments & list of all lncRNA extracted from Reference file.*
Output: *Location of lncRNA fragments constituting the real negative dataset from given CLIP-Seq experiments*

*INITIALIZE **all_pos_data**:=table containing all positive RNA fragments belonging to both lncRNA and mRNA of given CLIP-Seq experiments*
*INITIALIZE **lnc_pos_data**:=table containing all positive RNA fragments belonging to only lncRNA of given CLIP-Seq experiments*
*INITIALIZE **ref_data**:= table containing all lncRNA from Reference*
*INITIALIZE **final_neg_data**:= empty table will contain final set of generated negative data*

*FOR each RNA fragments R_i of **lnc_pos_data***
*INITIALIZE **length**=Length of RNA fragments R_i*
*INITIALIZE **left_interval**=10KB-1MB interval located left position from the start of R_i*
*INITIALIZE **right_interval**=10KB-1MB interval located right position from the end of R_i*
*ASSIGN **all_pos_sub_data**:= subset of **all_pos_data** based on given chromosome & strand of R_i*
*ASSIGN **ref_sub_data**:= subset of **ref_data** based on given chromosome & strand of R_i which also overlaps with **left_interval** & **right_interval*** from which negative data will be sampled.

*INITIALIZE **neg_data**:= empty table will contain start, end, chromosome and strand columns*
*FOR each lncRNA L_i of **ref_sub_data***
*ASSIGN **neg_start**:=random location between start and end of L_i*
*ASSIGN **neg_end**:=**neg_start+length** of corresponding RNA fragments R_i*
*ASSIGN **neg_chromosome**:=Chromosome of R_i*
*ASSIGN **neg_strand**:=Strand of R_i*
*APPEND row of **neg_data** table with **neg_start, neg_end, neg_chromosome, neg_strand***
END FOR

*FOR each generated negative data N_i of **neg_data***
*IF start and end interval of N_i overlaps with start and end interval any of **all_pos_sub_data***
*REJECT N_i and Remove from **neg_data** table*
ELSE
ACCEPT N_i
ENDIF
ENDFOR
*ASSIGN **neg_sel:=** randomly selected one negative data from the **neg_data** table*
*APPEND row of **final_neg_data** table with **neg_sel***
ENDFOR

(e) Retrieval of sequence information for positive and negative dataset:

We have extracted sequence information using R programming based **BSgenome** tool downloaded from **Bioconductor (30)** package manager. **BSgenome.Hsapiens.UCSC.hg38, BSgenome.Hsapiens.UCSC.hg19** and **BSgenome.Hsapiens.UCSC.hg18** packages have been deployed to extract the sequences corresponding to the hg38, hg19 and hg18 builds respectively based

on given chromosome number, strand information, start & end location. Corresponding to the negative dataset, nucleotide bases for very few fragments remained missing and yielded **N (**for hg19 and hg18 build), which would have an adverse effect on the training procedures for ML operations further. Hence while retrieving the sequence information, presence of **N,** within the sequences for the negative dataset**,** necessitated repeating step **(d)** until all the fragments constituting the negative dataset are devoid of missing nucleotide **N.**

The final dataset to be used for ML training is provided in **Table 3**.

Dataset Type	# of Data
Training Data	245501
Validation Data	27279
Unknown Test Data	9468

Table 3: *Data Dimension corresponding to Training, Validation and Unknown Test Data*

(f) Extraction of Protein Sequences:
The sequences of 33 proteins corresponding to different CLIP-Seq experiments have been extracted from the Uniprot database (https://www.uniprot.org/)

Machine Learning:
This module consists of 3 subparts: **Data Preprocessing:** which generates ML compatible dataset**; ML Model Creation & Validation:** build stacking classifiers and train them with the ML compatible datasets; **Hyper Parameter Optimization (HPO):** tune numerous hyper-parameters associated with ML models using **Optuna** tool in order to enhance model performance

Data Preprocessing: This consists of two steps viz. Feature Selection & Data Normalization in order to extract the final set of features
Feature Selection: Corresponding to both lncRNA and Protein sequences, features are created based on normalized count of nucleotide bases or amino acids described as follows:
lncRNA Feature Selection:
Selected features for lncRNA data are fundamentally embedded in their sequences containing strand specific information, normalized counts of unique nucleotides A, C, G, T; 2 contiguous nucleotides or di-nucleotides (AA, AC, AG, AT, …, etc.); 3 contiguous nucleotides (AAA, AAC, AAG, AAT, …, etc.) and 4 contiguous nucleotides (AAAA, AAAC, AAAG, AAAT, …, etc.); overall yielding 341 features as shown in **Table 4.**

Features Name	Number of Features
Strand	1
Normalized Counts of unique nucleotides A, C, G, T	4
Normalized Counts of 2 contiguous nucleotides (AA, AC, AG, AT, …, etc.)	16
Normalized Counts of 3 contiguous nucleotides (AAA,	64

AAC, AAG, AAT, …, etc.)	
Normalized Counts of 4 contiguous nucleotides (AAAA, AAAC, AAAG, AAAT, …, etc.)	256
Total Number of lncRNA Features	1+4+16+64+256=341

Table 4: *Detailed Feature Description extracted from raw lncRNA sequences from CLIP-seq dataset*

Protein Feature Selection:

Properties of amino acids are essential for generating the features from raw protein sequences. Here 20 primary amino acids and their important properties have been viz. **Hydropathy, Chemical, Charge and Polarity** along with **Volume** to be implemented in feature selection strategy. Most of the features (https://www.imgt.org/IMGTeducation/Aide-memoire/_UK/aminoacids/IMGTclasses.html) have been generated as specified by Pommié, C., et al (31). Amino acids can be classified into 3 types: **Hydrophobic (A, C, I, L, M, F, W, V), Neutral (G, H, P, S, T, Y) and Hydrophilic (R, N, D, Q, E, K)** corresponding to **Hydropathy** property based on Kyte-Doolitle scale (32). Using the normalized counts of single, di and tri **Hydropathy** grouping based strategies; total 39 features have been generated. Corresponding to the **Chemical, Charge and Polarity** based properties, amino acids are segregated into **Aliphatic (G, A, V, L, I, P), Aromatic (F, Y, W), Polar_Uncharged (S, T, N, Q), Sulpher_Contained (C, M), Positive_Negative_Charged (D, E, H, K, R).** Following the same procedure i.e. single, di and tri normalized counts, total 155 features have been generated. Finally, inclusion of **Volume** properties help us to classify the amino acids into 5 classes: **Very_Small (A, G, S), Small (N, D, C, P, T), Medium (Q, E, H, V), Large (R, I, L, K, M), Very_Large (F, W, Y)** ultimately resulting in generation of 155 more features following their normalized counts of occurrence**.** Besides, the normalized counts of individual amino acids additionally yields 20 more features resulting in an overall 369 feature. Accumulating set of all attributes from lncRNA and protein creates 710 features to be trained by ML models which have been detailed in **Table 5.**

Features Name	Number of Features
Normalized Counts of each amino acids	20
Defining Hydropathy based properties in terms of amino acids where hydrophobic=1, neutral=2 and hydrophilic=3; Normalized Counts of unique hydropathy group (3 features), 2 contiguous hydropathy group (9 features) and 3 contiguous hydropathy group (27 features); **hydrophobic = ['A', 'C', 'I', 'L', 'M', 'F', 'W', 'V']** **neutral = ['G', 'H', 'P', 'S', 'T', 'Y']** **hydrophilic = ['R', 'N', 'D', 'Q', 'E', 'K']**	3+9+27=39
Defining Chemical, Charge and Polarity based properties in terms of amino acids where aliphatic=1, aromatic=2,polar_uncharged=3, sulphur_contained=4 and positive_negative_charged=5; Normalized Counts of unique group (5 features), 2 contiguous group (25 features) and 3 contiguous group (125 features) **aliphatic=['G', 'A', 'V', 'L', 'I', 'P']**	5+25+125=155

aromatic= ['F', 'Y', 'W'] **polar_uncharged= ['S', 'T', 'N', 'Q']** **sulpher_contained = ['C', 'M']** **positive_negative_charged= ['D', 'E', 'H', 'K', 'R']**	
Defining Volume based properties in terms of amino acids where very_small=1, small=2 medium=3, large=4 and very_large=5; Normalized Counts of unique volume group (5 features), 2 contiguous volume group (25 features) and 3 contiguous volume group (125 features) **very_small=['A', 'G', 'S']** **small=['N', 'D', 'C', 'P', 'T']** **medium=['Q', 'E', 'H', 'V']** **large=['R', 'I', 'L', 'K', 'M']** **very_large=['F', 'W', 'Y']**	5+25+125=155
Total Number of features for Protein only	20+39+155+155=369
Total number of features for both lncRNA and protein	341+369=710

Table 5: *Detailed Feature Description extracted from raw protein sequences and cumulated number of lncRNA and protein features*

Data Normalization:

The set of features generated above, belong to varied ranges, which would prevent the ML models for smoother convergence. Normalization procedure would scale those diverse ranges such that they become comparable resulting in removal of unwanted bias corresponding to larger variables. Following equation (1) provides the formulation behind the normalization procedure.

$$\text{Normalized_X} = \frac{X - mean(X)}{std(X)} \quad (1)$$

We have implemented scikit-learn based StandardScaler method for normalizing the data (http://scikit-learn.org/stable/modules/generated/sklearn.preprocessing.StandardScaler.html) as provided in equation(1).

ML Model Creation & Validation:

Figure 4 showing the detailed flowchart corresponding to our ML model building phase where we have incorporated **Stacking Ensemble (StackingClassifier)** based model building architecture. This is achieved by incorporating various ML models across multiple layers. It follows more generalized approach by comparing with bagging (33) and boosting (34) strategies. Our model architecture have been segregated into two layers: **Layer 1** constitutes hyperparameter optimized models **LightGBM (35)**, **XGBoost (36)** & their variants whereas, **Layer 2** merges the output from previous layers base models using **Logistic Regression** based meta-learning technique(shown in Figure 4).

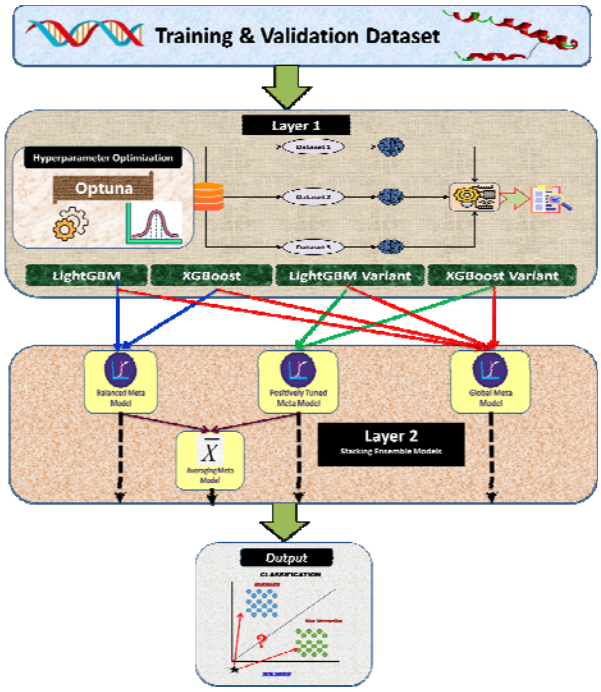


Figure 4: Machine learning model building

This figure elaborates the set of hierarchical layers associated with various machine learning models utilized in LncPTPred tool.

Layer 1 (Base Model): Layer 1 constitutes of 4 base models LightGBM, XGBoost and their corresponding variants. It has been found that both the general LightGBM & XGBoost models possess biasness towards non-interactive output, thus we have tweaked the associated weights a little bit in order to reduce such kind of biasness, resulting in formation of model variations.

Layer 2 (Stacking Ensemble Model): Layer 2 is also known as Stacking Ensemble layers, constructed by combining the prediction output extracted from the base model of previous layers and redirected to Logistic Regression which also acts our meta-learner to generate final prediction output. Here we have built four stacking meta-learner models by incorporating various combination of the base model as detailed in **Figure 4. Balanced Meta Classifier,** which merges the prediction output from general optimized LightGBM and XGBoost are fed into Logistic Regression meta-learner. **Positively Tuned Meta Classifier,** combine the outputs from variants of both LightGBM and XGBoost into the Logistic Regression meta-learner. **Averaging Meta Classifier** doesn't create any separate model, rather takes prediction probabilistic output from **Balanced Meta Classifier** & **Positively Tuned Meta Classifier.** It performs averaging operation and gives separate output. Finally, **Global Meta Classifier** combine prediction outputs from all of the 4 base models from Layer 1 i.e. LightGBM, XGBoost & their corresponding variants and send them to the Logistic Regression meta-learner in Layer 2.

Hyper Parameter Optimization (HPO): HPO depicts fine-tuning of various hyper-parameters associated with all kind of ML models. We have incorporated python based tool **Optuna (25)** which internally utilizes

Bayesian Optimization (37) by employing efficient sampling and pruning algorithm to fine tune the hyperparameters from dynamically constructed search space. This tool has been incorporated upon **LightGBM**, **XGBoost** and their variant models to optimize their respective hyper-parameters for execution of lncRNA-protein target prediction task.

Performance Metrics: In order to validate the efficacy of LncPTPred tool against various external tools, multiple performance metrics have been taken into consideration consisting of **Accuracy, Sensitivity, Specificity, F1 score, ROC-AUC** & **Confusion Matrix.** LncPTPred performs binary classification task having discrete target variable containing 1 and 0 annotating positive and negative interaction respectively.

Comparison with other Validated Models: In order to validate the efficacy of LncPTPred tool, we have compared the performance of our model against 3 tools **RPISeq, LPI-CNNCP** & **Capsule-LPI.** Non-availability of pre-trained models (https://github.com/plhhnu/LPI-HyADBS; https://github.com/mrzResearchArena/PyFeat/), deprecation of the python environment (13) limited us to include others in the comparison. Further the web-pages of some tools (10,12) are unavailable.
Constructed using traditional ML models, RPISeq is one of the well cited tools defined for the prediction task of lncRNA-Protein interaction consisting of Support Vector Machine (SVM) and Random Forest (RF) where the features are defined using 3-mer protein and 4-mer RNA extracted from the sequences. Rest of the two tools have incorporated deep learning based prediction model. LPI-CNNCP proposed the Convolution Neural Network (CNN) model to perform prediction task where the feature set was generated using copy padding trick to convert variable length sequences of lncRNA/protein into fixed length inputs, further incorporating one-hot encoding in order to transform CNN-compatible image data. Capsule-LPI tool embeds multimodal features in terms of secondary structure, motif, sequence and physiochemical properties where the Capsule network forms the basis of their prediction model. From the result section it can be easily visualized that LncPTPred completely outperforms these specified models.

Webserver Implementation and generating the output: Both **Python** & **R** programming languages have been incorporated in order to execute the entire **LncPTPred,** lncRNA-Protein target prediction task. The software packages associated with Python language (Version 3.8) have been downloaded using **Anaconda** (https://www.anaconda.com/) suite whereas **R** based packages have been downloaded using **cran** (https://cran.r-project.org/) and **Bioconductor** (https://www.bioconductor.org/) **(30)** tools. Sequences corresponding to raw RNA fragments obtained from CLIP experiments are generated using **Bioconductor** based **BSgenome** (https://bioconductor.org/packages/BSgenome/) package. In order to perform the entire data wrangling operation corresponding to target prediction task we have utilized both **pandas** (https://pandas.pydata.org/) (Version 1.3.3) and **numpy** (https://numpy.org/)(Version 1.19.5) tools. As per as the machine learning models are concerned we have utilized **scikit-learn** (https://scikit-learn.org/) (Version 1.0.2) package to execute data normalization, formation of stacking ensemble model and performance based metrics generation. **LightGBM** (https://LightGBM.readthedocs.io/) (Version 2.3.1), **XGBoost** (https://xgboost.readthedocs.io/en/stable/python/index.html) (Version 1.6.2) have been deployed for creation of ML models whereas, **Optuna** (https://optuna.org/) performs the hyperparameter optimization associated with those models. Data visualization has been encompassed by both **Matplotlib** (https://matplotlib.org/) in python and **ggplot2** (https://ggplot2.tidyverse.org/) in R. **Logomaker (38)** (https://logomaker.readthedocs.io/en/latest/) (Version 0.8) is utilized for sequence visualization in terms of sequence logo. We have utilized **HTML5, Javascript** and **PHP** to build the web-server.

In order to perform prediction analysis user needs to select protein names from given list & provide the lncRNA sequence. Window length section specifies the fragmented length of given lncRNA sequence where min value is 10, max value is 30 and default is 20 as determined by violin plot in **Figure 3** where length distribution of dataset mostly lies between 20 and 25. Shift size determines moving window length where default setting is considered to be 1, as our main aim is to include maximum overlapping window. Finally, the strand is to be considered as + / – .

Given the protein name, lncRNA sequence, window size, shift size and strand information provided by the user, the results are generated in following steps:

- LncRNA sequence is segmented according to the window size and shift size.
- Corresponding to each segment, if prediction probability≥0.5 predicted by at least 3 of the 4 models, it is considered as interacting segment else not.
- Finally, the overlapping segments are merged to get the **Final_Interacting_Score** which denotes the interaction probability corresponding to each of the binding segments of a particular lncRNA.

RESULTS & DISCUSSIONS

At first we have checked for specific sequence motifs (contained within the nucleotide sequence corresponding to the RNA binding proteins) which affects lncRNA's binding affinity. Thereafter we have performed a set of experiments to evaluate the performance of LncPTPred.

Specificity of an LncRNA target binding site: Previous reports suggest greater affinity of an lncRNA towards AU rich region for HuR (39), FUS (40) and AUF1(41). The positive interacting nucleotide sequences (corresponding to these RBPs) extracted from such RBP specific CLIP-Seq experiments were scanned and similar motifs were observed. **Figure 5** showing the sequence logo represents the predominance of AU(T)-rich region at the binding location of the 3 RBPs with the lncRNA segments.

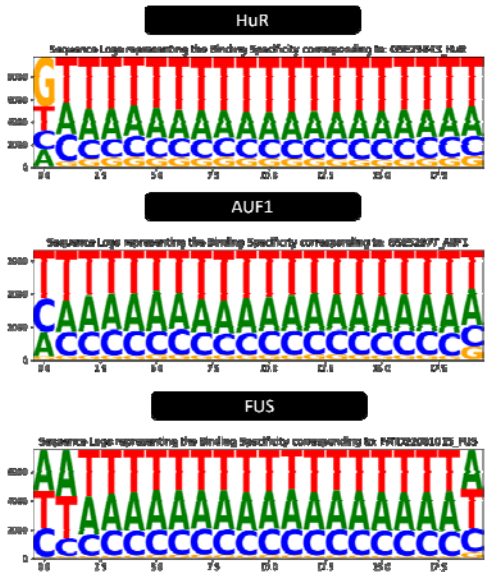
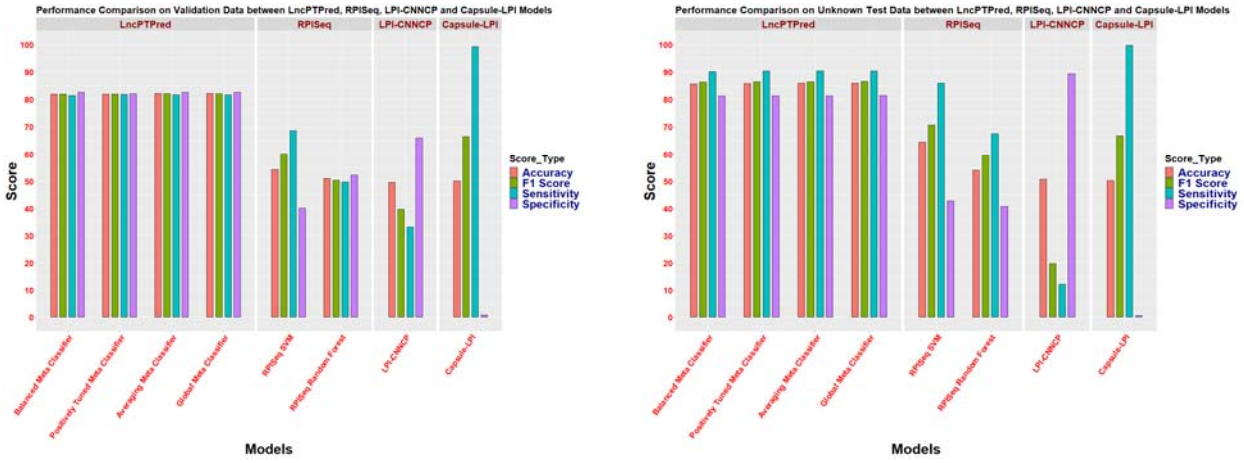


Figure 5: Sequence logo plot describing the binding specificity of lncRNAs with proteins

Logo plots showing the binding specificity corresponding to positive interacting lncRNA segments associated with 3 proteins HuR, AUF1 and FUS.

Performance of LncPTPred for lncRNA-protein target prediction: We have implemented stacking ensemble based ML models consisting of 4 separate classifiers **Balanced Meta Classifier, Positively Tuned Meta Classifier, Averaging Meta Classifier** & **Global Meta Classifier.** Various metrics have been utilized to measure the efficacy of LncPTPred tools, viz. **Accuracy, Sensitivity, Specificity, F1 score, ROC-AUC** & **Confusion Matrix.** In this paper we have compared the performance of the abovementioned 4 models used in **LncPTPred** with 2 models used in **RPISeq** viz. Support Vector Machine (SVM) and Random Forest (RF), **Capsule-LPI** & **LPI-CNNCP** tools using both validation and unknown test data. **Figure 6a & 6b** shows the bar diagram comparing the Accuracy, F1 score , Sensitivity and Specificity of LncPTPred models with RPISeq, LPI-CNNCP & Capsule-LPI models for validation data and unknown test data respectively.

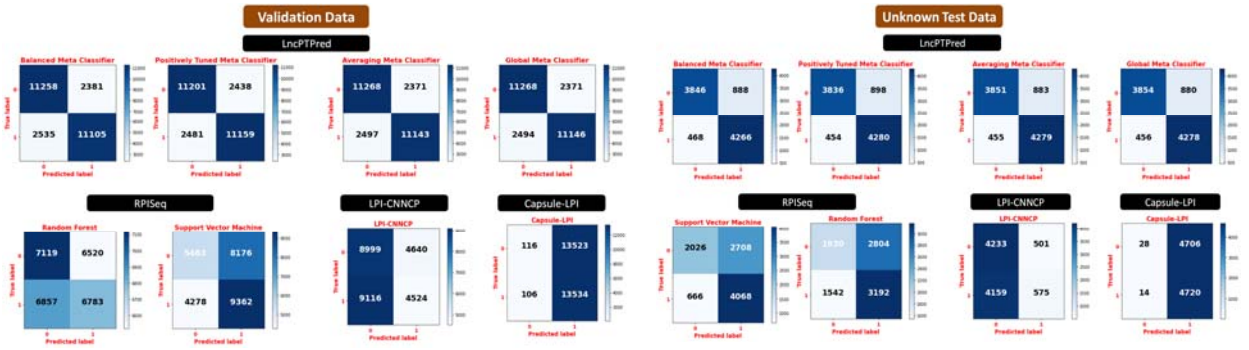


(a) (b)

Figure 6: Performance Comparison of the various ML Models used to develop LncPTPred tool with RPISeq, LPI-CNNCP and Capsule -LPI for (a) Validation and (b) Unknown Test data

Bar chart showing the performance comparison among various LncPTPred models (Balanced, Positively Tuned, Averaging & Global Meta Classifier) with RPISeq models (SVM and RF), LPI-CNNCP and Capsule-LPI in terms of Accuracy, F1 Score, Sensitivity and Specificity metrics corresponding to (a) Validation and (b) Unknown Test data.

Accuracy and F1 score of the models used for LncPTPred outperforms the other models for both the validation and unknown test datasets. On the contrary, sensitivity score of Capsule-LPI is 100 whereas its specificity score is 0 (for both the datasets) which reveals the biasness of Capsule-LPI towards positive prediction leading towards greater false positive outputs. Further, the specificity score of LPI-CNNCP is very high and sensitivity very low (for both the datasets) which reveals the biasness of LPI-CNNCP towards negative prediction leading towards greater false negative outputs. This is better revealed in the confusion matrix for validation and unknown test data provided in **Figure 7a & 7b.** Hence, considering the global metrics like Accuracy and F1-score, LncPTPred scores the highest as compared to LPI-CNNCP, Capsule-LPI and RPISeq**.**

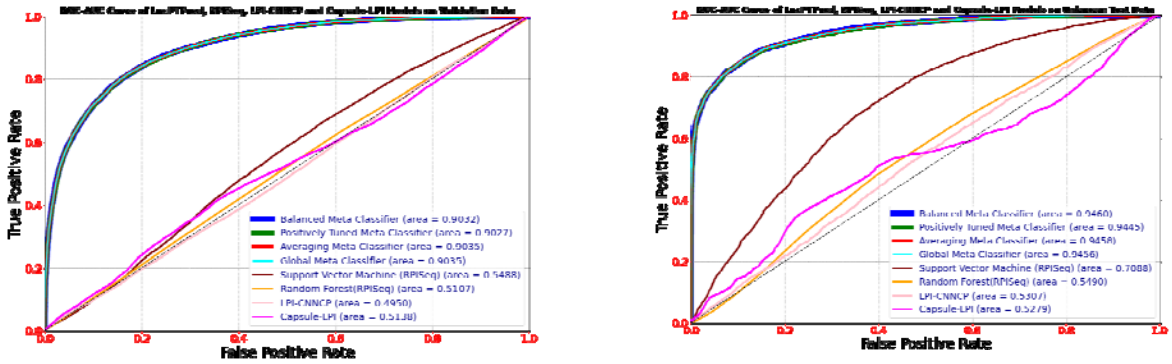


(a) **(b)**

Figure 7: Confusion Matrix showing comparison of Performance Statistics corresponding to LncPTPred tool with RPISeq, LPI-CNNCP and Capsule-LPI for (a) Validation and (b) Unknown Test data

This Matrix shows the True Positive & True Negative predictions representing the number of accurate predictions whereas False Positive & False Negative predictions representing the number of wrong predictions comparing LncPTPred tool (viz. Balanced, Positively Tuned, Averaging & Global Meta Classifier) with RPISeq (viz. SVM and RF), LPI-CNNCP and Capsule-LPI tools.

Same holds true for ROC-AUC curve (as shown in **Figure 8a & 8b)**, where the models used in LncPTPred covers maximum area (>0.9) for both validation and unknown test data as compared to that covered by other classifiers.



(a) **(b)**

Figure 8: ROC-AUC Plot for (a) Validation and (b) Unknown Test data

ROC-AUC curve depicting the performance of the classification models used for developing LncPTPred, RPISeq, LPI-CNNCP and CAPSULE-LPI corresponding to (a) Validation and (b) Unknown Test data

LncPTPred performance corresponding to disease specific lncRNA-protein interactions: We have incorporated LncPTPred tool in the context of disease specific lncRNA-protein target prediction. Here we

have considered experimentally validated disease specific lncRNA-protein target interactions tabulated in **Table 6**.

Protein	lncRNA	Disease	Pubmed ID
ALKBH5	PVT1	Osteosarcoma	PMID: 32021563
ALKBH5	PVT1	Lung Cancer	PMID: 36376862
AUF1	FILNC1	Renal Cell Carcinoma	PMID: 28978906
FUS	RMST	Glioblastoma	PMID: 32069702
SRSF1	DGCR5	Esophageal Squamous Cell Carcinoma	PMID: 34099633
YTHDC1	HOTAIR	Breast cancer	PMID: 36441764
ALKBH5	NEAT1	Colon Cancer	PMID: 32913527
CAPRIN1	MACC1-DT	Colorectal Cancer	PMID: 33762340
HuR	MALAT1	Breast cancer	PMID: 27197265
METTL14	XIST	Colorectal Cancer	PMID: 32111213
METTL3	MALAT1	Pancreatic Cancer	PMID: 36212476
G3BP1	LINC00472	Breast and Lung Cancer	PMID: 29588351
YTHDF2	MIAT	Cardiac Hypertrophy	PMID: 35383152
ZFP36	MBNL1-AS1	Breast cancer	PMID: 35518784
ZFP36	MCM3AP-AS1	Breast cancer	PMID: 35116304
FUS	SOX2-OT	Pancreatic Cancer	PMID: 34552054
LIN28B	H19	Pediatric Leukemia	PMID: 26969084
METTL3	LINC01605	Colorectal Cancer	PMID: 34544413
SRSF1	NEAT1	Glioma	PMID: 31200124
YTHDF2	NEAT1	Renal Cell Carcinoma	PMID: 34813676
ESR1	TMPO-AS1	Breast cancer	PMID: 31501276
HuR	FOXD3-AS1	Lung Cancer	PMID: 34605863
METTL3	FAM225A	Nasopharyngeal Carcinogenesis	PMID: 31331909
ALKBH5	TRERNA1	Diffuse Large B Cell Lymphoma	PMID: 35031597
FUS	KCNQ1OT1	Heart Failure	PMID: 32497620
HuR	MYClo-1	Colorectal Cancer	PMID: 25663692
MSI2	LINC00942	Gastric Cancer	PMID: 35073459
PTBP2	RBAKDN	Spermatogenesis Disruption	PMID: 34707642
WTAP	NORAD	Intervertebral Disc Degeneration	PMID: 35304463
YBX1	DSCAM-AS1	Cancer Progression	PMID: 32929382
YBX1	MILIP	Renal Cell Carcinoma	PMID: 36028903

Table 6: *Disease specific reported lncRNA-Protein interaction*

Most of these lncRNAs have multiple transcripts. LncPTPred predicts lncRNA-protein interactions with all these transcripts. Thereafter, binding score for each of the transcripts for a particular lncRNA is obtained from the percentage of interacting segments among all the available segments (based on the window size = 20 and shift size = 1). The lncRNA transcript (for a particular lncRNA-protein interaction) with the highest binding score has been finally depicted in the bar graphs of **Figure 9a-9d**.

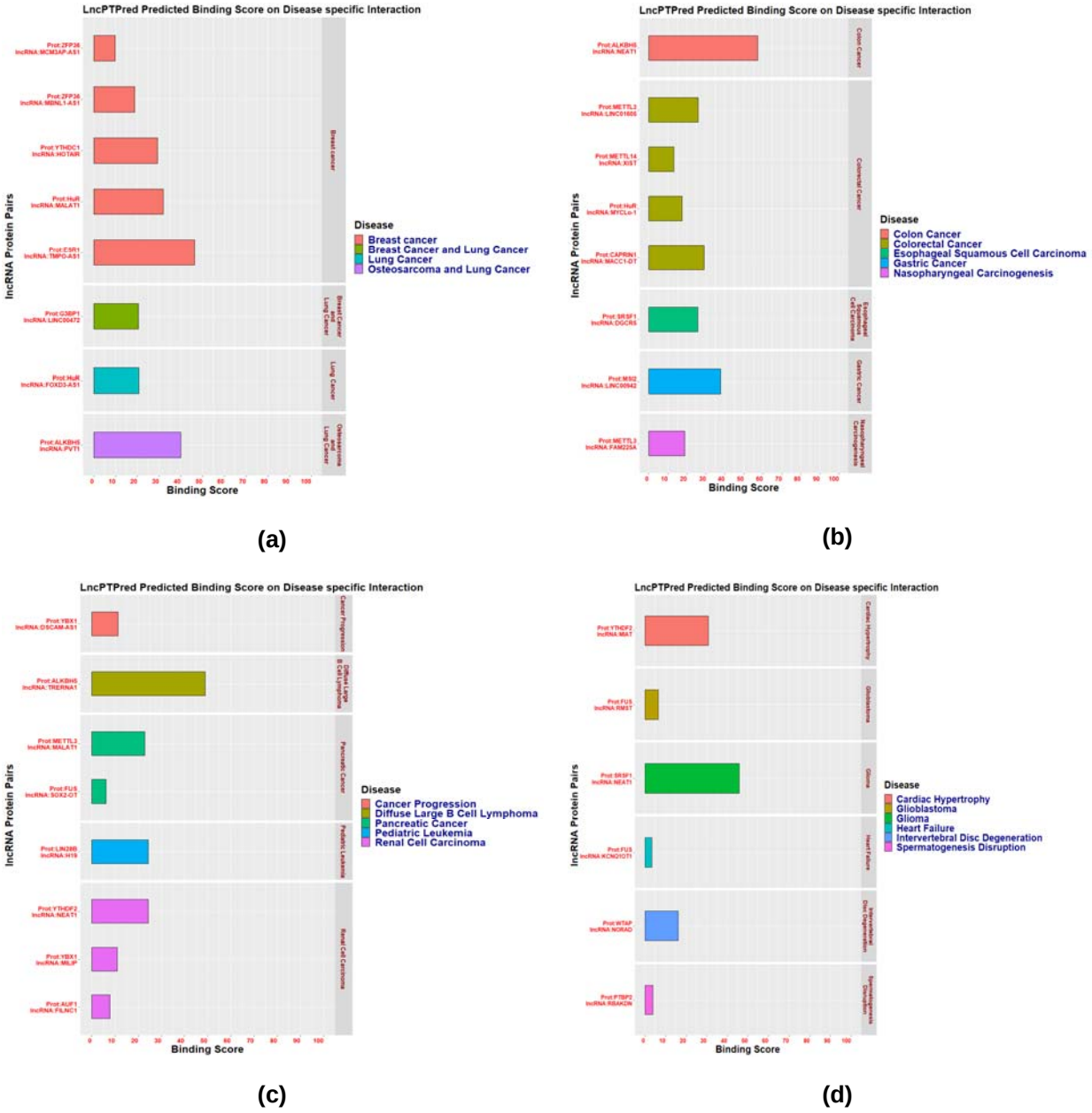
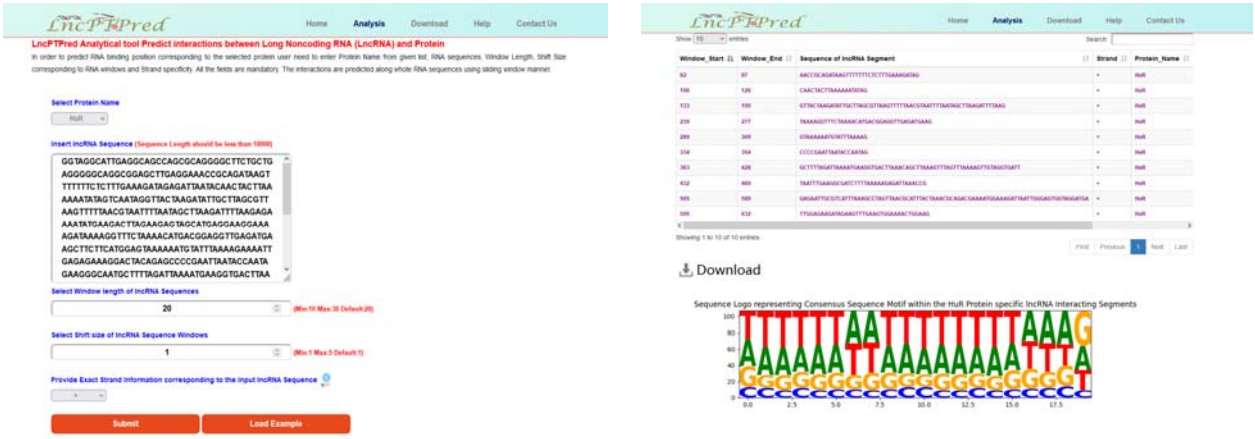


Figure 9: Performance of LncPTPred for predicting disease specific lncRNA-protein interactions

Horizontal Bar plot in figure (a)-(d) showing the lncRNA transcript with the highest binding score for a

particular lncRNA-protein interaction validated in case of various diseases.

Web Server Output: Figure 10a shows the user input page containing the input for lncRNA sequence, protein name and selection criteria regarding window size, shift size and strand specification. Once the user submits the query, it will provide the interacting segments and the **Final_Interacting_Score** as output. In order to show the binding specificity of the lncRNA segments, a sequence logo diagram is provided as shown in **Figure 10b**.



(a) **(b)**

Figure 10: LncPTPred Web Server

Snapshot detailing the graphical user interface of LncPTPred web server (a) showing the required input data to execute the tool and (b) the output containing interacting lncRNA segment details, sequence logo diagram and the Final_Interacting_Score

CONCLUSION

LncPTPred is an ML based target prediction tool involving lncRNA and protein. The biggest problem associated with this kind of target prediction scheme is the lack of real negative data. Recent reports on AI based lncRNA-protein target prediction algorithm relies on random shuffling techniques to tackle the issue ending up with noisy output resulting in most of the cases. We have tried to resolve the issue by considering CLIP-seq data. An extensive bioinformatics analysis have been done to extract sequence specific pattern associated with interacting positive data (corresponding to a particular protein) from within the CLIP-seq data. For that particular protein, the non-interacting segment served as the real negative data. We have considered Stacking Ensemble classifier as model architecture where Layer 1 consists of LightGBM, XGBoost & their variants as base model; the prediction output from the layer 1 have been merged using Logistic Regression meta-learner in Layer 2 to generate final output. Moreover we have enhanced the performance of our model by performing hyperparameter optimization using **Optuna** to set optimized values corresponding to model associated parameters. Finally, we have shown the efficacy of LncPTPred tool in two stages; comparing the performance of our models against RPISeq, LPI-CNNCP and Capsule-LPI tools where LncPTPred shows better performance against them. Moreover, we have showcased the efficiency of our model towards predicting some of the experimentally validated lncRNA-

protein interactions across multiple diseases. Unlike the other state-of-the-art prediction tools, LncPTPred provides the exact location of interaction which will provide a big support for designing validation experiments. Further, on availability of more CLIP-seq datasets, we plan to upgrade this tool by providing predictions for more than 33 proteins in future.

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DATA AVAILABILITY: The detail information about raw CLIP-Seq data can be extracted from **Table 1** & **Supplementary File 1.** The data files associated with training, validation and unknown test data utilized for ML task can be downloaded from either the webserver http://bicresources.jcbose.ac.in/zhumur/lncptpred/download.php or from the actual github location https://github.com/zglabDIB/lncptpred/tree/master/dataset

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DECLARATION OF COMPETING INTEREST: The authors have no competing interest to declare.

AUTHOR CONTRIBUTION: ZG hypothesized, conceptualized and provided overall supervision of the study. ZG and GD initially designed the experiments. Data curation and analysis was done by GD and TD. GD and ZG drafted the manuscript.

FIGURE AND TABLE LEGENDS

Figures:

Figure 1: Workflow of the entire work

The flow diagram depicts the various steps involved towards development of LncPTPred which includes Data Curation from CLIP-Seq data, development of the ML based models, output generation and data visualization

Figure 2: Data Curation Workflow

It depicts the pre-processing of the raw CLIP-seq data to build the training dataset, validation and unknown test dataset which includes the extraction of positive and real negative data. It also depicts the curation of protein sequence information

Figure 3: Length Distribution of Interacting lncRNA sequences extracted from CLIP-seq data

The violin plot shows the length distribution of interacting lncRNA sequences extracted from CLIP-seq data subdivided as training, validation and unknown test dataset which will serve as an important parameter for determining the window size of the search space to be selected by the users while using LncPTPred.

Figure 4: Machine learning model building

This figure elaborates the set of hierarchical layers associated with various machine learning models utilized in LncPTPred tool.

Figure 5: Sequence logo plot describing the binding specificity of lncRNAs with proteins

Logo plots showing the binding specificity corresponding to positive interacting lncRNA segments associated with 3 proteins HuR, AUF1 and FUS.

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Bar chart showing the performance comparison among various LncPTPred models (Balanced, Positively Tuned, Averaging & Global Meta Classifier) with RPISeq models (SVM and RF), LPI-CNNCP and Capsule-LPI in terms of Accuracy, F1 Score, Sensitivity and Specificity metrics corresponding to (a) Validation and (b) Unknown Test data.

Figure 7: Confusion Matrix showing comparison of Performance Statistics corresponding to LncPTPred tool with RPISeq, LPI-CNNCP and Capsule-LPI for (a) Validation and (b) Unknown Test data

This Matrix shows the True Positive & True Negative predictions representing the number of accurate predictions whereas False Positive & False Negative predictions representing the number of wrong predictions comparing LncPTPred tool (viz. Balanced, Positively Tuned, Averaging & Global Meta Classifier) with RPISeq (viz. SVM and RF), LPI-CNNCP and Capsule-LPI tools.

Figure 8: ROC-AUC Plot for (a) Validation and (b) Unknown Test data

ROC-AUC curve depicting the performance of the classification models used for developing LncPTPred, RPISeq, LPI-CNNCP and CAPSULE-LPI corresponding to (a) Validation and (b) Unknown Test data

Figure 9: Performance of LncPTPred for predicting disease specific lncRNA-protein interactions

Horizontal Bar plot in figure (a)-(d) showing the lncRNA transcript with the highest binding score for a particular lncRNA-protein interaction validated in case of various diseases.

Figure 10: LncPTPred Web Server

Snapshot detailing the graphical user interface of LncPTPred web server (a) showing the required input data to execute the tool and (b) the output containing interacting lncRNA segment details, sequence logo diagram and the Final_Interacting_Score

Tables:

Table 1: Input Training Dataset utilized for developing LncPTPred

Table 2: Unknown Test Dataset utilized to validate the performance of LncPTPred against external models.

Table 3: Data Dimension corresponding to Training, Validation and Unknown Test Data

Table 4: Detailed Feature Description extracted from raw lncRNA sequences from CLIP-seq dataset

Table 5: Detailed Feature Description extracted from raw protein sequences and cumulated number of lncRNA and protein features

Table 6: Disease specific reported lncRNA-Protein interaction

SUPPLEMENTARY FILE LEGEND

Supplementary File 1: Detailed Information about PAR-CLIP & HITS-CLIP experiments considered for building the training, validation and unknown test dataset compatible for ML task.