GENE FINDING REVISITED: IMPROVED ROBUSTNESS THROUGH STRUCTURED DECODING FROM LEARNING EMBEDDINGS

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Paper under double-blind review

ABSTRACT

Gene finding is the task of identifying the locations of coding sequences within the vast amount of genetic code contained in the genome. With an ever increasing quantity of raw genome sequences, gene finding is an important avenue towards understanding the genetic information of (novel) organisms, as well as learning shared patterns across evolutionarily diverse species. The current state of the art are graphical models usually trained per organism and requiring manually curated data sets. However, these models lack the flexibility to incorporate deep learning representation learning techniques that have in recent years been transformative in the analysis of protein sequences, and which could potentially help gene finders exploit the growing number of sequenced genomes to expand performance across multiple organisms. Here, we propose a novel approach, combining learned embeddings of raw genetic sequences with exact decoding using a latent conditional random field. We show that the model achieves performance matching the current state of the art, while increasing training robustness, and removing the need for manually fitted length distributions. As language models for DNA improve, this paves the way for more performant cross-organism gene-finders.

1 INTRODUCTION

Genes are patches of deoxyribonucleic acid (DNA) in our genome that encode functional and structural products of the cell. The central dogma of biology states that these segments are transcribed into ribonucleic acid (RNA) and in many cases translated into the amino acid sequences of proteins. In recent years, the machine learning community has dedicated considerable attention specifically to studying proteins, and solving various protein-related tasks, with the aid of deep learning. This focus has resulted in impressive advances within the field (Detlefsen et al., 2022; Jumper et al., 2021; Rao et al., 2020; Shin et al., 2021). Less attention has been paid to the DNA sequences themselves, despite the fact that finding genes in a genome remains an important open problem. Due to technological advances, the rate by which genomes are sequenced is rising much more rapidly than we can reliably annotate genes experimentally, and without proper gene annotations, we lack information about the proteins encoded in these sequences. In particular, for taxonomies that are sparsely characterised or highly diverse, such as fungi, this hinders us from extracting essential information from newly sequenced genomes.

The wealth of available genomic data suggests that this is an area ripe for a high-capacity deep learning approaches that automatically detect the most salient features in the data. This potential has in recent years been clearly demonstrated in the realm of proteins where deep learning has proven extremely effective in both the supervised setting (Alley et al., 2019; Hsu et al., 2022; Jumper et al., 2021) and in the unsupervised setting (Rao et al., 2021; 2020; Vig et al., 2021). In particular, embeddings obtained in transformer based protein language models have pushed the boundaries for performance in many downstream sequence-based prediction tasks. The advantages of such models are two-fold: 1) they enable pre-training in cases where unlabelled data far outweighs labelled data and 2) they have demonstrated the ability to learn across diverse proteins. We are currently witnessing an emerging interest in language models for DNA as well, but progress in this area has proven more difficult than for its protein counterpart. In a completely unsupervised setting the amount of DNA data is orders of magnitude larger than that of proteins, and the signals are

correspondingly sparse. For instance, Eukaryotic genomes consist of millions to billions of DNA base pairs but only a small percentage are genes and an even smaller percentage codes for protein (approx. 1% in the human genome). Genes also have an intricate structure which places demands on a high degrees of consistency between output labels predicted at different positions in the sequence. In particular, genes contain both coding segments (called CDS or exon) and intron segments. Only the coding segments are retained in the final protein, while the introns are removed. The process in which introns are removed is called splicing, which occurs after the gene is transcribed from DNA to RNA. After introns are spliced out, the RNA is translated to amino acid sequences (the protein product). Each amino acid is encoded by a codon (a triplet of DNA nucleotides) in the RNA sequence. Due to this codon structure, the annotation of the coding sequences in the gene must be extremely accurate, as shifting the frame of the codon with just one will result in a nonsensical protein. Gene prediction thus consists of correctly annotating the boundaries of a gene as well as the intron splice sites (donor/acceptor sites), a task challenged both by the imbalanced data but also by the extreme accuracy needed.

The current state-of-the-art in gene-finding relies on Hidden Markov Models (HMMs) and exact decoding (e.g. Viterbi) to ensure the required consistency among predictions at different output positions. To make these methods work well in practice, considerable effort has been put into hand-coded length distributions inside the HMM transition matrix, and a careful curation of the training data to ensure that the length statistics are representative for the genome in question. The resulting HMM models have been highly successful and have dominated the field for more than a decade. However, they are generally difficult to train, and have no mechanism for incorporating learned embeddings and context dependent learned length distributions. The goal with this paper is to develop a new approach that is compatible with contemporary deep learning practices, can be trained without manual feature engineering and careful data curation, while maintaining the capability for exact decoding.

Here we present an approach to gene prediction that is able to both incorporate prior knowledge of gene structure in the form of a latent graphs in a Conditional Random Fields as well as embeddings learned directly from the DNA sequence. This approach proves easy to train naively while still achieving high performance across a range of diverse genomes. We highlight that the resulting model is very flexible and open to improvement either by including external hints or by improvement of the current DNA sequence embeddings. We benchmark against three other supervised algorithms (Augustus, Snap, GlimmerHMM) and find that the performance of our model competes with that of the state-of-the-art (Scalzitti et al., 2020) without a strong effort put into model-selection. However, as pre-trained DNA models start to emerge and improve we expect that the full potential of this approach will be realised.

2 Related Work

Current gene prediction algortihms are Hidden Markov Models (HMM) or Generalized HMMs. These include Augustus (Stanke & Waack, 2003) , Snap (Korf, 2004)., GlimmerHMM (Majoros et al., 2004) and Genemark (Ter-Hovhannisyan et al., 2008). With the exception of Genemark, all these models are trained fully supervised and on a per-organism basis. Genemark is similarly trained on one organism but in an iterative self-supervised manner. In practice, gene prediction is often done through meta-prediction pipelines such as Braker (Hoff et al., 2016), Maker2 (Holt & Yandell, 2011) and Snowyowl (Reid et al., 2014), which typically combine preexisting HMM models with external hints (e.g. protein or RNA alignments) and/or iterated training.

Focusing on the individual gene predictors, Augustus is currently the tool of choice in the supervised setting, according to a recent benchmark study (Scalzitti et al., 2020). It is an HMM model with explicit length distributions for introns and CDS states. The model also includes multiple types of intron and exon states emitting either fixed-length sequences or sequences from a length distribution given by a specific choice of self-transition between states. This intron model has been shown to be key to its performance; without it Augustus was found to be incapable of modelling length distributions in introns correctly Stanke & Waack (2003). Models like Snap and GlimmerHMM follow similar ideas, but differ in their transition structure. In particular, GlimmerHMM includes a splice site model from Genesplicer (Pertea et al., 2001).

These HMM-gene predictors are known to be sensitive to the quality of the input data set. For instance, the Augustus training guidelines specifies requirements for non-redundancy, non-overlapping genes and restrictions to single transcripts per genes. These considerations are not merely theoretical. In the preparation of the baseline results for this paper, we have attempted to re-train several of these models on our own data set splits, but were unable to obtain competitive results. The Augustus guidelines also report diminishing returns for data sets with more than 1000 genes, and highlighting the importance of quality over quantity in the training set in this scenario. These recommendation are sensible in a low-data regime, but we might wish to relax them as we obtain more genomic data. It would be convenient if we could train gene predictor models on lower quality data sets, including multiple, potentially conflicting transcripts arising for instance from alternative splicing. The goal of this paper is to explore modelling strategies towards this goal.

Eventually, we would also like to train cross-genome gene predictors, for instance using featurizations obtained from pre-trained language models. Following the success of pre-trained models on protein sequences, DNA language modelling is starting to emerge as a field. Currently, two such models are available: DNABert (Ji et al., 2021) and GPN (Benegas et al., 2022). Both are trained on a single organism, each across a whole genome with a sequence window of 512 nucleotides. While DNABert is a standard transformer based model for which long sequences can be computationally prohibitive, GPN (Benegas et al., 2022) is based on dilated convolutions where it could be possible to extend the sequence length. In this work we perform preliminary explorations of using GPN embeddings for gene prediction.

3 METHODS

3.1 MODEL

Latent conditional random fields Genomic datasets are highly unbalanced, containing only a very small percentage of the donor and acceptor labels. Since identification of these labels are paramount for correct annotation, the model must be designed to deal robustly with this. Furthermore, we wish to encode prior knowledge of gene structure in order to produce consistent predictions. Finally, the model must be flexible in the type of input used, so that context dependent embeddings can be learned directly from the sequence. If the raw nucleotide sequence is used, input takes the onehot encoded values $\mathbb{X} = \{A, C, G, T, N\}$, where N is unknown nucleotides. Output as well as ground truth gene annotations are of the same length as the input, labelled per position according to the label sets in Appendix A.1.

To fulfil these requirements a Linear chain Conditional Random Field (CRF) (Lafferty et al., 2001) model was chosen. The CRF can, like the HMM, encode gene structure in its graph, but because it is the conditional rather than the joint probability is modelled and the independence assumptions are less strict than in an HMM, it is possible to model flexible context dependencies. It also allows training a feature embedding model simultaneously.

Similar to the strategy followed by HMM gene predictors, we impose structure on the transition weight potentials of the graph in the CRF, ensuring that only biologically meaningful transitions are permitted. The choice of graph is flexible and depend on what prior knowledge is to be incorporated. To encode gene structure in the graph we restrict allowed transitions to sets of hidden states according to the knowledge we wish to impose. One important restriction is allowing only transitions according to codon structure. This is done by expanding the all label states to a set of three denoting the position in the codon:

$$E = \{E_1, E_2, E_3\}$$

The remainder of the label states must also be expanded so the codon position is tracked in the intron, ensuring that the final reading frame and length of the prediction is consistent with a protein coding gene. Label sets and their descriptions can be found i Appendix A.1.

In order to model the gene structure in the graphs without having to encode it directly in the data, we introduce a set of hidden states from which the simpler set of observed labels are emitted (Morency et al., 2007). By introducing these hidden states we can now model the graph and its transitions as latent. This reduces the work of laboriously encoding a large label set but also the risk of mislabelling if the data quality is low. We can now leave it to the model to infer labels by learning their

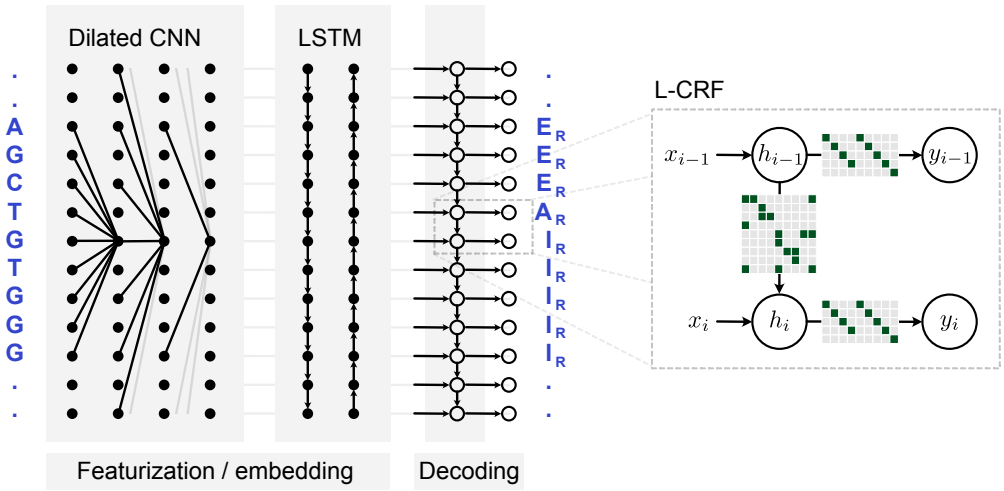

Figure 1: Overview of the model. The model takes a onehot encoded sequences. Here raw DNA sequence are shown but it can also potentially take tokenized sequences, alternatively the featurization can be replaced with an entirely different embedding model. The current feature model processes the input first with several dilated convolutional layers with residual connections and thereafter a bi-LSTM. The feature model outputs un-normalized label probabilities per positions which are then processed by the latent CRF to output label sequences in a manner consistent with the allowed transitions and emissions.

sequence dependent context directly from the input. The standard formulation of the CRF:

$$P(\mathbf{y}|\mathbf{x},\theta) = \frac{1}{Z}\exp\sum_{i=n}^{N}\left(T_{y_{n-1},y_n} + \theta(x_n)\right) \tag{1}$$

Where T is the learned transition weights of the observed labels and θ the learned input mapping. The partition function Z is the summed score over all possible label sequences:

$$Z = \sum_{y'\in Y}\exp\sum_{i=n}^{N}\left(T_{y'_{n-1},y'_n} + \theta(x_n)\right) \tag{2}$$

In a Latent CRF this changes to include a summation of the set of hidden states emitting a given observed state: (Lafferty et al., 2001):

$$P(\mathbf{y}|\mathbf{x},\theta) = \frac{1}{Z}\exp\sum_{i=n}^{N}\left(\sum_{h'\in\mathbb{H}_{y_{n-1}}}\sum_{h\in\mathbb{H}_{y_n}}(T_{h',h} + E_{h,y_n}) + \theta(x_n)\right) \tag{3}$$

Where $\mathbb{H}_{y_{n-1}}$ denotes the set of hidden states emitting the value taken by y_{n-1}. The learned transition matrix T is now over the hidden states and the emission matrix E denotes the emission weights from hidden to observed states. similarly the partition function now also sum over the set of hidden states that emits y:

$$Z = \sum_{y'\in Y}\exp\sum_{i=n}^{N}\left(\sum_{h'\in\mathbb{H}_{y_{n-1}}}\sum_{h\in\mathbb{H}_{y_n}}(T_{h',h} + E_{h,y_n}) + \theta(x_n)\right) \tag{4}$$

From here on CRF will be used interchangeably with Latent CRF.

Although the emission parameters E can also be learned, it is also possible to impose prior knowledge here. We formulate the allowed emissions in the strictest sense by restricting them to be binary,

with an emission E_{h_j,y_j} between h_j and y_i either allowed or forbidden. We also restrict the hidden states to be disjoint sets mapping only to one observed labels (see Appendix A.3 for allowed emissions between different sets of hidden and observed states). The majority of the experiments performed here use a graph which encodes codon structure as well as direction (see adjacency matrix Figure A.2). As we know that gene structure is similar on the forward and reverse strands we share the learned transition weights across direction. Figure 3.1 shows a full overview of our model.

Feature Model Embeddings In a standard CRF a set of user determined feature functions are calculated from the input for each position to model context dependencies. A weighting between them is learned by the CRF. Rather than weighted functions we use a feature model that learns a weighted, context dependent mapping from the onehot encoded input sequence to label to unnormalised label probabilities in the latent dimension. The specific choice of model is very flexible but the choice of architecture can affect how the context dependencies are learned. Here, we primarily use Long-Short Term Memory (LSTM) and Dilated Convolutions (CNN).

Training The model is trained with gradient descent on the Negative Log Likelihood function (NLL)
Inference To find the best label sequence under the model Viterbi decoding is used. Alternatively sampling can be performed according to the label probabilities, on the either across the entire sequence or for segments. Decoding is performed one the hidden states in order to preserve the graph structure. It is also possible to perform inference in the observed dimension which would enable maximising according to the summed marginal probabilities of each disjoint set of latent states (see Morency et al. (2007)). Posterior marginal probabilities per position are calculated with the forward-backward algorithm and can be used to asses model certainty for specific positions.

Choice of inference labels and metrics The label used for inference set contains the splice sites (donor and acceptor) as well as information about the gene direction (see Appendix A.1)

Accurately predicting the direction, as well as the donor and acceptor sites (i.e. start and end of introns), is highly important for predicting the correct protein. For this reason Matthews Correlation Coefficient as well as Macro weighted F1 score is reported a the label set containing exon/CDS, intron, donor and acceptor labels

4 Experiments

4.1 Data

Sequence and general feature format (gff3) annotation files are obtained for each genome as follows: Human - Gencode, Aspergillus Nidulans and Saccharomyces Cerevisiae - EnsembleFungi, Arabidopsis Thaliana - EnsemblPlants, C. Elegans Wormbase Frankish et al. (2021); Cunningham et al. (2022); Howe et al. (2017).

No augmentation or inspection of the data was performed, with the exception of ensuring the total length of CDS segments in each gene was divisible by 3, ensuring that valid protein product was possible. Gene annotations were extracted with a random flank of varying length (1-2000 nucleotides for most experiments shown). The data set was split randomly into train, validation and test sets, though ensuring that no gene was duplicated and the isoforms of the same gene was either not present or in the same set. To reduce compute time data set gene length was capped at 5000 or 10000 nucleotides. The test sets were used for benchmarking. The length of the flank can influence the performance depending on the inference task. For some ablation experiments simpler feature models have been chosen in order to reduce compute, these results may therefore reflect only a relative performance rather than the full potential.

4.2 Benchmark Algorithms

We benchmark our method against three other widely used gene prediction software (Augustus, Snap and GlimmerHMM). Given the sensitivity of these methods to the training sets, pre-trained parameter set for each species was used where available. This means we cannot rule out that sequences in our test set are part of the training set of the baseline models. Our reported results are thus a conservative estimate of our relative performance.

Table 1: Feature model ablations on A. Thaliana

FEATURE MODEL	**MCC**	**F1**
Linear-CRF (simple graph)	0.15	0.11
Linear-CRF (codon graph)	0.34	0.19
LSTM	0.79	0.75
LSTM-CRF	0.86	0.84
DilCNN-LSTM-CRF	0.89	0.85
GPN-CRF	0.74	0.75

4.3 LEARNED EMBEDDINGS IMPROVE PREDICTIONS

To explore the performance contribution of different aspects of our model we performed ablations, where parts of the model were excluded or simplified part of it (Table 1). Note that these models were trained on a smaller set of the *A. Thaliana* data set.

First, to test the capabilities of the CRF alone, a single linear layer is implemented to map each position from the one-hot encoded DNA sequence to the hidden states. Two graphs of different complexity were used for this experiment (see Figure A.2 and A.2 for graphs). While both Linear-CRFs exhibit very poor predictive power, the more complex codon graph clearly performs better that the simple graph, illustrating the need for a higher graph complexity to improve modelling performance. This is consistent with similar observations made for HMM-based gene predictors Stanke & Waack (2003).

By introducing a feature model such as an LSTM, performance is greatly enhanced. While an LSTM trained alone also displays decent performance it is evident from Figure A.5 that without the CRF, splice sites are often not labelled correctly which is vital for producing consistent predictions.

Interestingly training a linear layer on fixed embeddings from the GPN language model (Benegas et al., 2022) achieves high performance (Figure A.5). This indicates that the masked pre-training of the GPN model captures DNA sequence patterns relevant for gene prediction, despite only having trained on a single organism and primarily on non-coding DNA. The emergent field of DNA language modelling could have a significant effect on downstream DNA modelling tasks, as they have had in the protein field. These ablation studies reveal that with a good feature model, there is less need for as complex a graph structure. The feature model can instead learn complex dependencies directly from the sequence.

The best performing model uses a feature model with a combination of dilated convolutions as well as a bidirectional LSTM. We hypothesise that the increased performance over a LSTM alone is due to better long range information provided by the dilated convolutions. From here on we refer to this particular model as GeneDecoder (see architecture in Appendix A.4).

4.4 LEARNED EMBEDDINGS ACCURATELY MODEL LENGTH DISTRIBUTIONS

Accurately capturing length distributions of introns and exons is essential for a well performing gene predictor. Along with explicitly modelling exon length distributions, Stanke & Waack (2003) had to introduce an intron sub-model with states modelling both explicit length distributions as well as fixed length emissions, to meaningfully capture intron lengths.

We find that our model readily learns these signals directly from data (Figure (4.4)). The latent CRF without learned embedding is not sufficient to reproduce the true lengths resulting in a flattened distribution similar to Stanke & Waack (2003). However, with the learned embeddings, GeneDecoder captures the length distributions faithfully, especially for exons. Interestingly, we find that the Linear-GPN-CRF model does not capture length distributions better than a simple Linear layer, despite significantly outperforming it in Table 1).

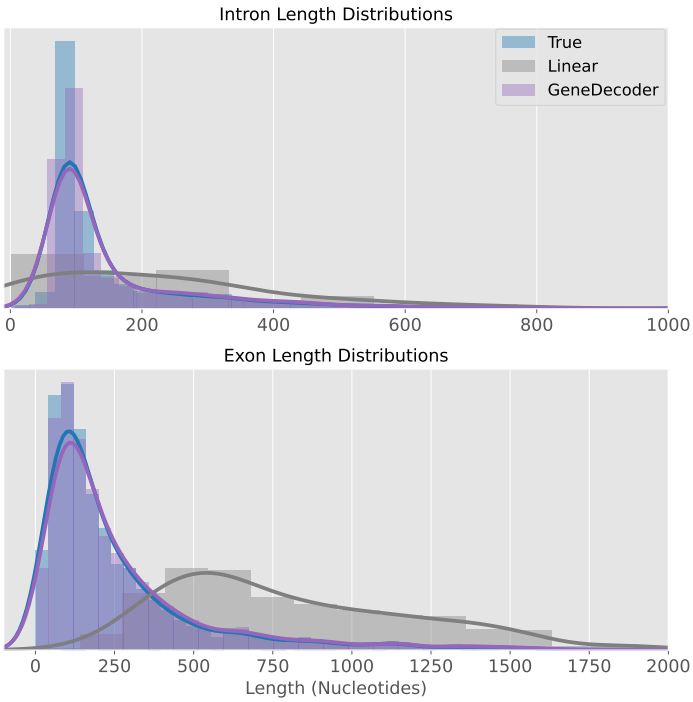

Figure 2: Learned embeddings capture length distributions of more accurately

4.5 Latent structure can infer unobserved labels

To test the capacity of the latent graph of our model we explore whether it can learn unobserved states directly from the sequence. One such example is inferring the directionality of genes. Genes can be encoded on both strand of the double-helical DNA but usually only one strand is sequenced. Genes that lay in the "reading" direction of sequenced strand are designated as forward and genes that would be read on the opposite strand as reverse. We train our model on a directionless set of observed labels (see *simple-labels-DA* in Appendix A.1). Since the the training data does not provide any information about directionality, the model must learn this directly from the sequence to be able to distinguish between directions.

Figure 4.4 4 shows the comparison between our model trained on the directionless label set as well as a set including directional labels (see *simple-direction-DA* in Appendix A.1). Although there is a reduction in performance between the two training regiments, the model is still able to learn and infer direction. Surprisingly the reduced performance does not originate from confusion between forward and reverse labels but rather due to the models propensity to over predict non-coding labels (see A.5).

4.6 Gene finding across evolutionary diverse species

To demonstrate the performance of our approach we benchmark against three widely used programs on five phylogenetically diverse well-studied species typically used in benchmarking (Table 2). Our model, GeneDecoder, was trained separately on genes from each organism. For the other models, pre-trained versions of the programs were used in order to not reduce performance by re-training on a data set that was insufficiently curated for their model. This benchmark limited and specific to predicting a gene within a nucleotide sequence, focusing on correctly labelling the splice sites.

We find that only Augustus achieves similar performance to our model , with Snap performing significantly lower across all species we it tested on. GlimmerHMM shows better performance but it is still has significantly lower predictive power compared to Augustus. This further cements previous findings that Augustus achieves state of the art performance.

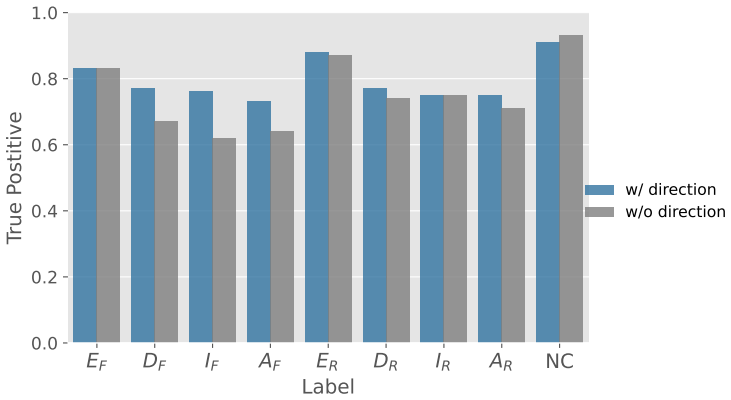

Figure 3: Inferring unobserved labels directional labels

Table 2: Comparison of algorithms

Model	**Human**		**A. Nidulans**		**A. Thalaiana**		**S. Cerevisiae**		**C. Elegans**	
	MCC	F1	MCC	F1	MCC	F1	MCC	F1	MCC	F1
GeneDecoder	**0.81**	**0.83**	**0.89**	**0.80**	**0.90**	**0.91**	0.88	0.46	**0.90**	**0.92**
Augustus	0.78	0.81	0.88	**0.80**	0.87	0.88	**0.89**	**0.58**	0.86	0.89
Snap	0.36	0.41	N/A	N/A	0.54	0.54	0.85	0.45	0.55	0.58
GlimmerHMM	0.74	0.76	N/A	N/A	0.77	0.78	N/A	N/A	0.78	0.80

Our model, GeneDecoder competes with or outperforms Augustus, which is a state-of-the-art gene prediction software, across the benchmark, with the exception of the lower F1 score for *S. Cerevisiae*. The low F1 score for S. Cerevisiae across all algorithms is due to the low number of intron-containing genes. Only 0.04% genes contain introns in the data set for this organism. It was not possible to obtain Augustus' training data set for this species, but according to the official training instructions of the software, data sets must be curated to contain a high proportion of intron-containing genes. This would explain the lower F1 score for GeneDecoder, however it also highlights that our model is not highly influenced by the quality of the data set.

We further compare Augustus with our model by training and testing on the original Augustus data sets for *Human* (Stanke, 2004). The training data set consists of 1284 training genes which is not ideal for deep learning settings. Furthermore, the Augustus data set is strictly homology reduced, allowing no more than 80% sequence similarity at the protein level. Nevertheless, our models closely matches the performance of Augustus on it own data set A.5 but while improving Augustus performance requires the use of external hints chosen by the user, our model may be improved by learning better mebeddings.

5 Conclusion

Here we present a novel approach, GeneDecoder, to gene prediction combining the advantages of graphical models with the flexibility and potential of deep learned embeddings.

We find that the Latent CRF gives us not only the ability to preserve the graph-encoding of prior knowledge, as in the state-of-the-art HMMs gene predictors, but also offers an advantage by allowing us to learn embeddings directly from the sequence. This advantage is not only visible in the performance, but also in the low effort required to construct data sets and train the model. The greatest advantage, however, might well be the great flexibility of the learned embeddings and the potential to improve them as the DNA modelling field advances.

Even now this seems evident from the preliminary studies performed here using embeddings from a DNA language model trained only on a single species, with very short input sequences. We expect that as the field progresses language models more specifically tailored to DNA will emerge, modelling longer range interactions and training across multiple species. Such results have already been demonstrated by the success of the Enformer model (Avsec et al., 2021), which demonstrated the importance of long range information as well as the ability to learn downstream tasks that were not trained on.

One potential limitation of our model is that the amount of non-coding DNA (in our cases the flanking regions) in the training data set can affect the performance of the model on data with significantly more non-coding DNA. This issue can be resolved in the training process by scaling the training to larger data sets containing longer non-coding regions. As this presents a technical challenge, along with a time and resource challenge, rather than a scientific one we leave it for future work. Our model provides the capacity to include external hints in the inference process. Either via sampling high probability pathways around fixed labels but also via modification of the hidden state probabilities. The latter method can even be used as a strategy during training, highlighting the flexibility of out model. Lastly, model per position certainty is directly provided model in the form of posterior marginal probabilities. We leave it for future exploration to rigorously benchmark this.

We also expect that language modelling will alleviate issues like this by modelling far better and more informative embeddings of the raw sequence. In this regard we view the model presented here as an initial iteration that can only improve as DNA modelling does. We anticipate that future iterations of GeneDecoder will incorporate some form of self-supervised pre-training across various species which could vastly improve not only prediction tasks in species with rich gene annotation data but also in novel or understudied organisms.

6 CODE AVAILABILITY

Code will be available on github upon publication.

REFERENCES

Ethan C. Alley, Grigory Khimulya, Surojit Biswas, Mohammed AlQuraishi, and George M. Church. Unified rational protein engineering with sequence-based deep representation learning. *Nature methods*, 16(12):1315–1322, December 2019. ISSN 1548-7091. doi: 10.1038/s41592-019-0598-1. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7067682/.

Žiga Avsec, Vikram Agarwal, Daniel Visentin, Joseph R. Ledsam, Agnieszka Grabska-Barwinska, Kyle R. Taylor, Yannis Assael, John Jumper, Pushmeet Kohli, and David R. Kelley. Effective gene expression prediction from sequence by integrating long-range interactions. *Nature Methods*, 18(10):1196–1203, October 2021. ISSN 1548-7105. doi: 10.1038/s41592-021-01252-x. URL https://www.nature.com/articles/s41592-021-01252-x. Number: 10 Publisher: Nature Publishing Group.

Gonzalo Benegas, Sanjit Singh Batra, and Yun S. Song. DNA language models are powerful zero-shot predictors of non-coding variant effects. preprint, Bioinformatics, August 2022. URL http://biorxiv.org/lookup/doi/10.1101/2022.08.22.504706.

Fiona Cunningham, James E Allen, Jamie Allen, Jorge Alvarez-Jarreta, M Ridwan Amode, Irina M Armean, Olanrewaju Austine-Orimoloye, Andrey G Azov, If Barnes, Ruth Bennett, Andrew Berry, Jyothish Bhai, Alexandra Bignell, Konstantinos Billis, Sanjay Boddu, Lucy Brooks, Mehrnaz Charkhchi, Carla Cummins, Luca Da Rin Fioretto, Claire Davidson, Kamalkumar Dodiya, Sarah Donaldson, Bilal El Houdaigui, Tamara El Naboulsi, Reham Fatima, Carlos Garcia Giron, Thiago Genez, Jose Gonzalez Martinez, Cristina Guijarro-Clarke, Arthur Gymer, Matthew Hardy, Zoe Hollis, Thibaut Hourlier, Toby Hunt, Thomas Juettemann, Vinay Kaikala, Mike Kay, Ilias Lavidas, Tuan Le, Diana Lemos, José Carlos Marugán, Shamika Mohanan, Aleena Mushtaq, Marc Naven, Denye N Ogeh, Anne Parker, Andrew Parton, Malcolm Perry, Ivana Piližota, Irina Prosovetskaia, Manoj Pandian Sakthivel, Ahamed Imran Abdul Salam, Bianca M Schmitt, Helen Schuilenburg, Dan Sheppard, José G Pérez-Silva, William Stark, Emily Steed, Kyösti Sutinen,

Ranjit Sukumaran, Dulika Sumathipala, Marie-Marthe Suner, Michal Szpak, Anja Thormann, Francesca Floriana Tricomi, David Urbina-Gómez, Andres Veidenberg, Thomas A Walsh, Brandon Walts, Natalie Willhoft, Andrea Winterbottom, Elizabeth Wass, Marc Chakiachvili, Bethany Flint, Adam Frankish, Stefano Giorgetti, Leanne Haggerty, Sarah E Hunt, Garth R IIsley, Jane E Loveland, Fergal J Martin, Benjamin Moore, Jonathan M Mudge, Matthieu Muffato, Emily Perry, Magali Ruffier, John Tate, David Thybert, Stephen J Trevanion, Sarah Dyer, Peter W Harrison, Kevin L Howe, Andrew D Yates, Daniel R Zerbino, and Paul Flicek. Ensembl 2022. *Nucleic Acids Research*, 50(D1):D988–D995, January 2022. ISSN 0305-1048. doi: 10.1093/nar/gkab1049. URL https://doi.org/10.1093/nar/gkab1049.

Nicki Skafte Detlefsen, Søren Hauberg, and Wouter Boomsma. Learning meaningful representations of protein sequences. *Nature Communications*, 13(1):1914, April 2022. ISSN 2041-1723. doi: 10.1038/s41467-022-29443-w. URL https://www.nature.com/articles/s41467-022-29443-w. Number: 1 Publisher: Nature Publishing Group.

Adam Frankish, Mark Diekhans, Irwin Jungreis, Julien Lagarde, Jane E Loveland, Jonathan M Mudge, Cristina Sisu, James C Wright, Joel Armstrong, If Barnes, Andrew Berry, Alexandra Bignell, Carles Boix, Silvia Carbonell Sala, Fiona Cunningham, Tomás Di Domenico, Sarah Donaldson, Ian T Fiddes, Carlos García Girón, Jose Manuel Gonzalez, Tiago Grego, Matthew Hardy, Thibaut Hourlier, Kevin L Howe, Toby Hunt, Osagie G Izuogu, Rory Johnson, Fergal J Martin, Laura Martínez, Shamika Mohanan, Paul Muir, Fabio C P Navarro, Anne Parker, Baikang Pei, Fernando Pozo, Ferriol Calvet Riera, Magali Ruffier, Bianca M Schmitt, Eloise Stapleton, Marie-Marthe Suner, Irina Sycheva, Barbara Uszczynska-Ratajczak, Maxim Y Wolf, Jinuri Xu, Yucheng T Yang, Andrew Yates, Daniel Zerbino, Yan Zhang, Jyoti S Choudhary, Mark Gerstein, Roderic Guigó, Tim J P Hubbard, Manolis Kellis, Benedict Paten, Michael L Tress, and Paul Flicek. GENCODE 2021. *Nucleic Acids Research*, 49(D1):D916–D923, January 2021. ISSN 0305-1048. doi: 10.1093/nar/gkaa1087. URL https://doi.org/10.1093/nar/gkaa1087.

Katharina J. Hoff, Simone Lange, Alexandre Lomsadze, Mark Borodovsky, and Mario Stanke. BRAKER1: Unsupervised RNA-Seq-Based Genome Annotation with GeneMark-ET and AUGUSTUS. *Bioinformatics*, 32(5):767–769, March 2016. ISSN 1367-4803. doi: 10.1093/bioinformatics/btv661. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6078167/.

Carson Holt and Mark Yandell. MAKER2: an annotation pipeline and genome-database management tool for second-generation genome projects. *BMC Bioinformatics*, 12(1):491, December 2011. ISSN 1471-2105. doi: 10.1186/1471-2105-12-491. URL https://doi.org/10.1186/1471-2105-12-491.

Kevin L. Howe, Bruce J. Bolt, Myriam Shafie, Paul Kersey, and Matthew Berriman. WormBase ParaSite - a comprehensive resource for helminth genomics. *Molecular and Biochemical Parasitology*, 215:2–10, July 2017. ISSN 0166-6851. doi: 10.1016/j.molbiopara.2016.11.005. URL https://www.sciencedirect.com/science/article/pii/S0166685116301608.

Chloe Hsu, Hunter Nisonoff, Clara Fannjiang, and Jennifer Listgarten. Learning protein fitness models from evolutionary and assay-labeled data. *Nature Biotechnology*, 40(7):1114–1122, July 2022. ISSN 1546-1696. doi: 10.1038/s41587-021-01146-5. URL https://www.nature.com/articles/s41587-021-01146-5. Number: 7 Publisher: Nature Publishing Group.

Yanrong Ji, Zhihan Zhou, Han Liu, and Ramana V Davuluri. DNABERT: pre-trained Bidirectional Encoder Representations from Transformers model for DNA-language in genome. *Bioinformatics*, 37(15):2112–2120, August 2021. ISSN 1367-4803, 1460-2059. doi: 10.1093/bioinformatics/btab083. URL https://academic.oup.com/bioinformatics/article/37/15/2112/6128680.

John Jumper, Richard Evans, Alexander Pritzel, Tim Green, Michael Figurnov, Olaf Ronneberger, Kathryn Tunyasuvunakool, Russ Bates, Augustin Žídek, Anna Potapenko, Alex Bridgland, Clemens Meyer, Simon A. A. Kohl, Andrew J. Ballard, Andrew Cowie, Bernardino Romera-Paredes, Stanislav Nikolov, Rishub Jain, Jonas Adler, Trevor Back, Stig Petersen, David Reiman,

Ellen Clancy, Michal Zielinski, Martin Steinegger, Michalina Pacholska, Tamas Berghammer, Sebastian Bodenstein, David Silver, Oriol Vinyals, Andrew W. Senior, Koray Kavukcuoglu, Pushmeet Kohli, and Demis Hassabis. Highly accurate protein structure prediction with AlphaFold. *Nature*, 596(7873):583–589, August 2021. ISSN 1476-4687. doi: 10.1038/s41586-021-03819-2. URL https://www.nature.com/articles/s41586-021-03819-2. Number: 7873 Publisher: Nature Publishing Group.

Ian Korf. Gene finding in novel genomes. *BMC Bioinformatics*, 5(1):59, May 2004. ISSN 1471-2105. doi: 10.1186/1471-2105-5-59. URL https://doi.org/10.1186/1471-2105-5-59.

John D. Lafferty, Andrew McCallum, and Fernando C. N. Pereira. Conditional Random Fields: Probabilistic Models for Segmenting and Labeling Sequence Data. In *Proceedings of the Eighteenth International Conference on Machine Learning*, ICML '01, pp. 282–289, San Francisco, CA, USA, June 2001. Morgan Kaufmann Publishers Inc. ISBN 978-1-55860-778-1.

W. H. Majoros, M. Pertea, and S. L. Salzberg. TigrScan and GlimmerHMM: two open source ab initio eukaryotic gene-finders. *Bioinformatics*, 20(16):2878–2879, November 2004. ISSN 1367-4803, 1460-2059. doi: 10.1093/bioinformatics/bth315. URL https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/bth315.

Louis-Philippe Morency, Ariadna Quattoni, and Trevor Darrell. Latent-Dynamic Discriminative Models for Continuous Gesture Recognition. In *2007 IEEE Conference on Computer Vision and Pattern Recognition*, pp. 1–8, Minneapolis, MN, USA, June 2007. IEEE. ISBN 978-1-4244-1179-5 978-1-4244-1180-1. doi: 10.1109/CVPR.2007.383299. URL http://ieeexplore.ieee.org/document/4270324/.

Mihaela Pertea, Xiaoying Lin, and Steven L. Salzberg. GeneSplicer: a new computational method for splice site prediction. *Nucleic Acids Research*, 29(5):1185–1190, March 2001. ISSN 0305-1048. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC29713/.

Roshan Rao, Joshua Meier, Tom Sercu, Sergey Ovchinnikov, and Alexander Rives. Transformer protein language models are unsupervised structure learners. preprint, Synthetic Biology, December 2020. URL http://biorxiv.org/lookup/doi/10.1101/2020.12.15.422761.

Roshan M. Rao, Jason Liu, Robert Verkuil, Joshua Meier, John Canny, Pieter Abbeel, Tom Sercu, and Alexander Rives. MSA Transformer. In *Proceedings of the 38th International Conference on Machine Learning*, pp. 8844–8856. PMLR, July 2021. URL https://proceedings.mlr.press/v139/rao21a.html. ISSN: 2640-3498.

Ian Reid, Nicholas O'Toole, Omar Zabaneh, Reza Nourzadeh, Mahmoud Dahdouli, Mostafa Abdellateef, Paul MK Gordon, Jung Soh, Gregory Butler, Christoph W. Sensen, and Adrian Tsang. SnowyOwl: accurate prediction of fungal genes by using RNA-Seq and homology information to select among ab initio models. *BMC Bioinformatics*, 15(1):229, July 2014. ISSN 1471-2105. doi: 10.1186/1471-2105-15-229. URL https://doi.org/10.1186/1471-2105-15-229.

Nicolas Scalzitti, Anne Jeannin-Girardon, Pierre Collet, Olivier Poch, and Julie D. Thompson. A benchmark study of ab initio gene prediction methods in diverse eukaryotic organisms. *BMC Genomics*, 21(1):293, April 2020. ISSN 1471-2164. doi: 10.1186/s12864-020-6707-9. URL https://doi.org/10.1186/s12864-020-6707-9.

Jung-Eun Shin, Adam J. Riesselman, Aaron W. Kollasch, Conor McMahon, Elana Simon, Chris Sander, Aashish Manglik, Andrew C. Kruse, and Debora S. Marks. Protein design and variant prediction using autoregressive generative models. *Nature Communications*, 12(1):2403, April 2021. ISSN 2041-1723. doi: 10.1038/s41467-021-22732-w. URL https://www.nature.com/articles/s41467-021-22732-w. Number: 1 Publisher: Nature Publishing Group.

Mario Stanke. Augustus: datasets, January 2004. URL http://bioinf.uni-greifswald.de/augustus/datasets/. Publisher: Institute for Mathematics and Computer Science, University of Greifswald.

Mario Stanke and Stephan Waack. Gene prediction with a hidden Markov model and a new intron submodel. *Bioinformatics*, 19(suppl_2):ii215–ii225, September 2003. ISSN 1367-4803. doi: 10.1093/bioinformatics/btg1080. URL https://doi.org/10.1093/bioinformatics/btg1080.

Vardges Ter-Hovhannisyan, Alexandre Lomsadze, Yury O. Chernoff, and Mark Borodovsky. Gene prediction in novel fungal genomes using an ab initio algorithm with unsupervised training. *Genome Research*, 18(12):1979–1990, December 2008. ISSN 1088-9051, 1549-5469. doi: 10.1101/gr.081612.108. URL https://genome.cshlp.org/content/18/12/1979. Company: Cold Spring Harbor Laboratory Press Distributor: Cold Spring Harbor Laboratory Press Institution: Cold Spring Harbor Laboratory Press Label: Cold Spring Harbor Laboratory Press Publisher: Cold Spring Harbor Lab.

Jesse Vig, Ali Madani, Lav R. Varshney, Caiming Xiong, Richard Socher, and Nazneen Fatema Rajani. BERTology Meets Biology: Interpreting Attention in Protein Language Models, March 2021. URL http://arxiv.org/abs/2006.15222. arXiv:2006.15222 [cs, q-bio].

A Appendix

A.1 Label sets

Generally five types of labels are used:

E : Exon/CDS (i.e. the coding part of a sequence)

I : Intron

D : Donor site (one of the two splice sites)

A : Donor site (one of the two splice sites)

NC : Non-coding or intergenic regions of the genome.

Subscript F and R (e.g. E_F and E_F) are used to denote the forward and reverse direction of the gene. Numbered subscripts are used to denote codon structure (i.e. $E_{1,F}$ is the first exon nucleotide in a codon in the forward direction).

Simple-DA:

The simplest set of states used.

$$\mathbb{Y} = \Big\{E, D, I, A, NC\Big\}$$

Simple-direction-DA:

Expands the set of states to include information about the direction/strand of the gene.

$$\mathbb{Y} = \Big\{E_F, D_F, I_F, A_F, E_R, E_R, I_R, A_R, NC\Big\}$$

Codon-direction-DA:

Further expands the set states to include not only direction but also codon structure.

$$\mathbb{Y} = \Big\{E_{1,F}, E_{2,F}, E_{3,F}, D_{1,F}, D_{2,F}, D_{3,F}, I_{1,F}, I_{2,F}, I_{3,F}, A_{1,F}, A_{2,F}, A_{3,F},$$
$$E_{1,R}, E_{2,R}, E_{3,R}, D_{1,R}, D_{2,R}, D_{3,R}, I_{1,R}, I_{2,R}, I_{3,R}, A_{1,R}, A_{2,R}, A_{3,R}, NC\Big\}$$

A.2 Graphs

Graphs are represented as adjacency matrices and are named according to the label sets described in Appendix A.1. A grey cell signifies a forbidden transition while the weights of the green cells are learned by model.

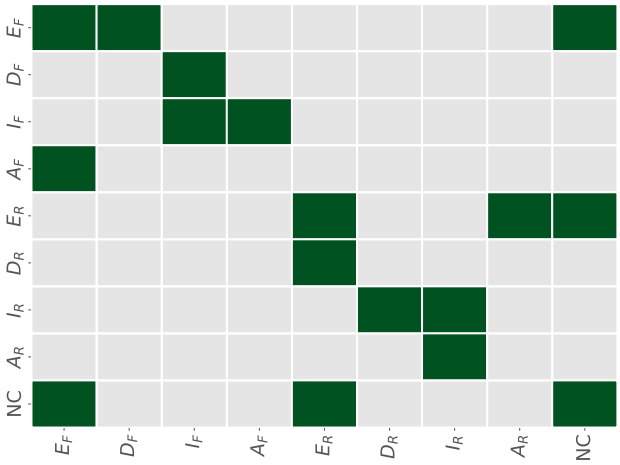

Figure 4: Graph of allowed transitions for *simple-direction-DA* labels

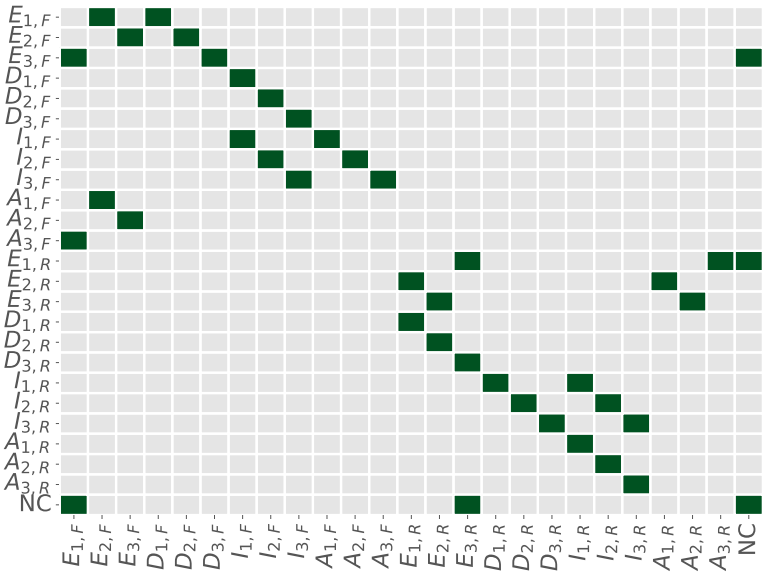

Figure 5: Graph of allowed transitions for *codon-direction-DA* labels

A.3 EMISSIONS

A grey cell signifies a forbidden emission while the weights of the green cells are set to 1 (i.e. an allowed emissions).

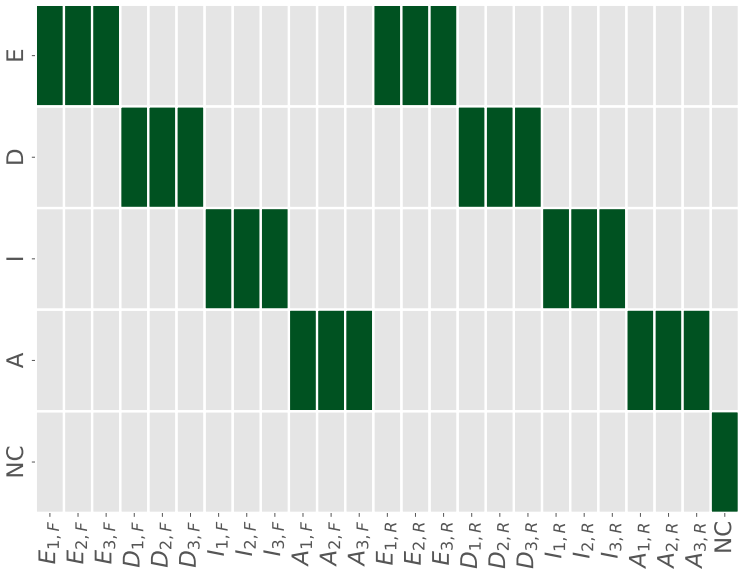

Figure 6: Allowed emissions from *codon-direction-DA* labels to *simple-DA* labels

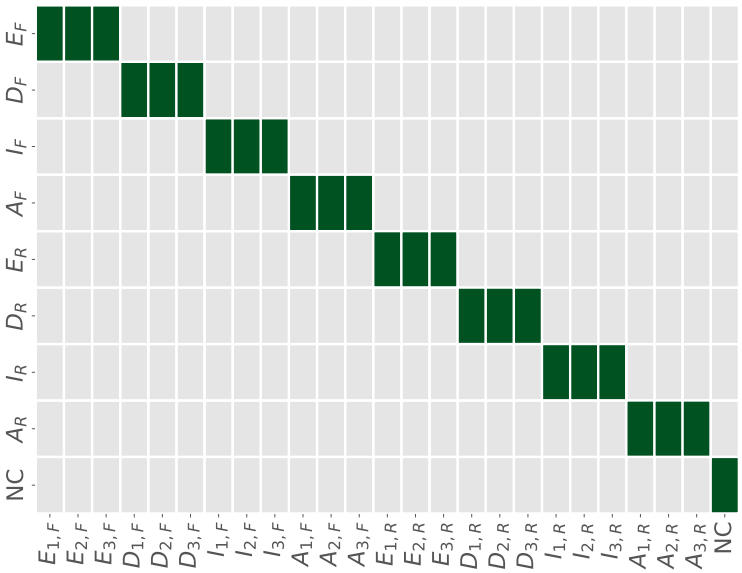

Figure 7: Allowed emissions from *codon-direction-DA* labels to *simple-direction-DA* labels

A.4 GENEDECODER FEATURE MODEL ARCHITECHTURE

Feature Model

Where $|\mathbb{X}|$ is the number of channels in the input, and $|\mathbb{H}|$ is the size of the set of hidden states. In most cases $\mathbb{X} = \{A, C, G, T, N\}$.

DilatedCNN(

Layer 1 : Conv1d(in channels $= |\mathbb{X}|$, out channels $= 50$, kernel size $= (9,)$, stride $= 1$, dilation $= 1$), ReLU

Layer 2 : Conv1d(in channels $= 50$, out channels $= 50$, kernel size $= (9,)$, stride $= 1$, dilation $= 2$), ReLU

Layer 3 : Conv1d(in channels $= 50$, out channels $= 50$, kernel size $= 9,)$, stride $= 1$, dilation $= 4$), ReLU

Layer 4 : Conv1d(in channels $= 50$, out channels $= 50$, kernel size $= 9,)$, stride $= 1$, dilation $= 8$), ReLU

Layer 5 : Conv1d(in channels $= 50$, out channels $= 50$, kernel size $= 9,)$, stride $= 1$, dilation $= 16$), ReLU

Layer 6 : Conv1d(in channels $= 50$, out channels $= 50$, kernel size $= 9,)$, stride $= 1$, dilation $= 32$), ReLU

)

LSTM(

LSTM layer : lstm(50, hidden layers $= 100$, bidirectional $=$ True)

Output layer : linear(input size $= 200$, output size $= |\mathbb{H}|$, bias $=$ True,

dropout(0.2)), ReLU

)

A.5 SUPPLEMENTARY FIGURES

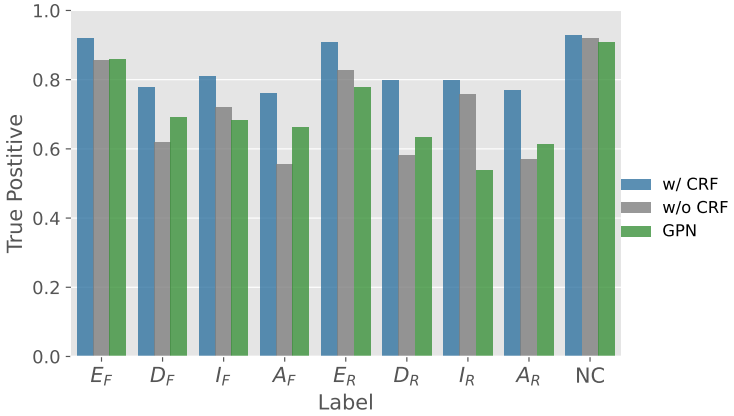

Figure 8: Comparison of true positive rate per label for a LSTM-CRF, LSTM and Linear-GPN-CRF model respectively.

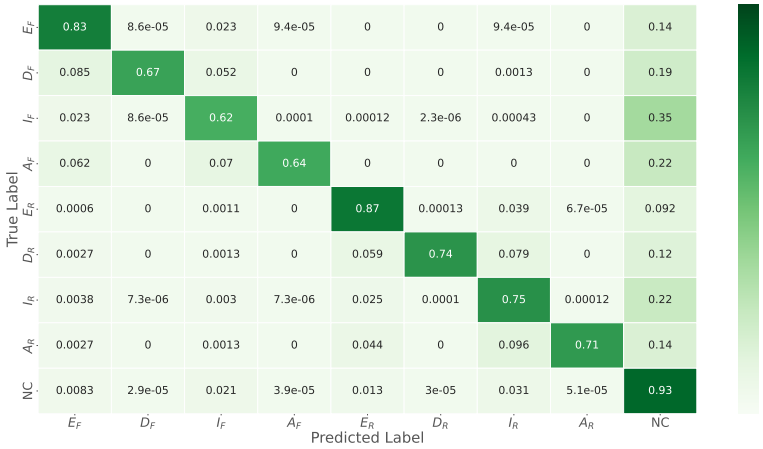

Figure 9: Confusion matrix for inference of direction on test set performance of model trained on *simple-DA* label set

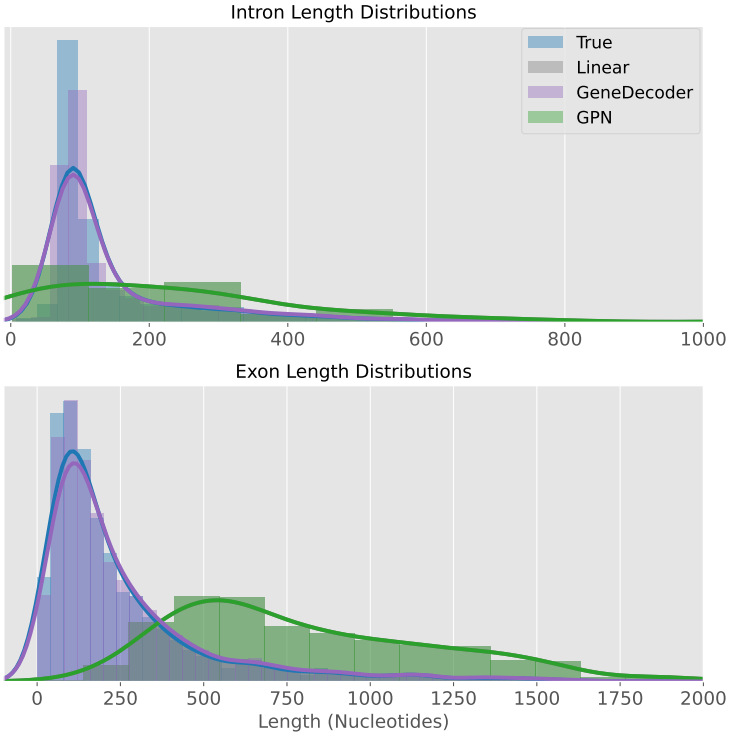

Figure 10: True and predicted length distributions of introns and exons for *A. thaliana*

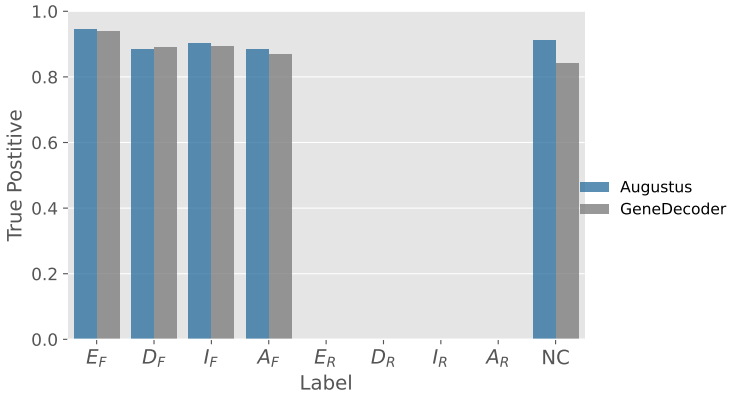

Figure 11: True positive rate per label for training and testing on the original Augustus *Human* datasets