PLEK: a tool for predicting long non-coding RNAs and messenger RNAs based on an improved k-mer scheme

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- Long non-coding RNAs (lncRNAs, typically >200 nt), are of particular interest because they contribute to many important biological processes;
- It remains a challenge to distinguish mRNAs from lncRNAs;
- LncRNAs show many features similar to mRNAs, such as poly(A) tails, splicing and approximate sequence length.

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- Several tools, such as CPC and PhyloCSF, have been developed based on known protein databases, intrinsic sequence features and sequence conservation properties.
- A tool named Coding-Non-Coding Index (CNCI) was developed. It discriminates coding from non-coding transcripts using intrinsic sequence features.

Objective

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PLEK takes calibrated k-mer frequencies of a transcript sequence as its computational features. With these features, the support vector machine (SVM) algorithm was used to build a binary classification model to separate lncRNAs from mRNAs.

Data description

Human protein-coding transcripts were downloaded from the **RefSeq** database (release 60) and human long non-coding transcripts were collected from **GENCODE v17**.

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There were $34,691$ protein-coding transcripts with the length of >200 nt in the human **RefSeq** dataset, and $22,389$ long (>200 nt) non-coding transcripts in the human **GENCODE** dataset.

Improved k-mer scheme

Approach: k-mer usage and sliding-windows with a one-nucleotide step-length to analyze each transcript.

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For $k = 1$ to 5, had $4 + 16 + 64 + 256 + 1024 = 1,364$ patterns: 4 one-mer patterns, 16 two-mer patterns, 64 three-mer patterns, 256 four-mer patterns, and $1,024$ five-mer patterns.

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Sliding-window of length $k, k = 1, 2, ..., 5$, which slides along the transcript of length l by a step-length of one nucleotide

$$f_i = \frac{c_i}{s_k} w_k,\ \ k = 1, 2, 3, 4, 5.\ i = 1, 2, \ldots, 1364 \tag{1}$$

$$s_k = l - k + 1,\ \ k = 1, 2, 3, 4, 5 \tag{2}$$

$$w_k = \frac{1}{4^{5-k}},\ \ k = 1, 2, 3, 4, 5 \tag{3}$$

Construction of classification model

To produce a balanced training dataset, we collected all the 22,389 long non-coding transcripts from the **GENCODE** v17 dataset (labelled as the "negative" class) and randomly selected 22,389 protein-coding transcripts from the human **RefSeq** dataset (labelled as the "positive" class).

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Hyper Parameters: Optimal C of the SVM and gamma of the kernel were obtained using the grid search.

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Hyper Parameters: Optimal C of the SVM and gamma of the kernel were obtained using the grid search.
Validation: 10-fold cross-validation.

Simulation of indel sequencing errors

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A tool robust to such errors is desirable to distinguish lncRNAs and mRNAs, and facilitates annotation of lncRNAs and mRNAs of a species without whole-genome sequences.

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A tool robust to such errors is desirable to distinguish lncRNAs and mRNAs, and facilitates annotation of lncRNAs and mRNAs of a species without whole-genome sequences.
Simulated 0 to 3 single-base indel sequencing errors per 100 bases (the error rate p was 0% to 3%).

Construction of a real sequencing dataset

The first dataset was recently released by PacBio and, the second dataset, a HelaS3 cell line transcriptome, was sequenced by a 454 GS FLX Titanium platform.

Different usage frequencies of k-mer strings

Calculated the calibrated usage frequencies of all the 1,364 k-mer patterns in the positive training dataset (22,389 protein-coding transcripts) and negative training dataset (22,389 long non-coding transcripts)

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Wilcox rank-sum test was used to determine which k-mer pattern usage was significantly different between mRNAs and lncRNAs.

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Wilcox rank-sum test was used to determine which k-mer pattern usage was significantly different between mRNAs and lncRNAs.
With a significance level of 10^{-6}, was found that 1,278 patterns were significantly different in their usage

Performance in cross-species prediction

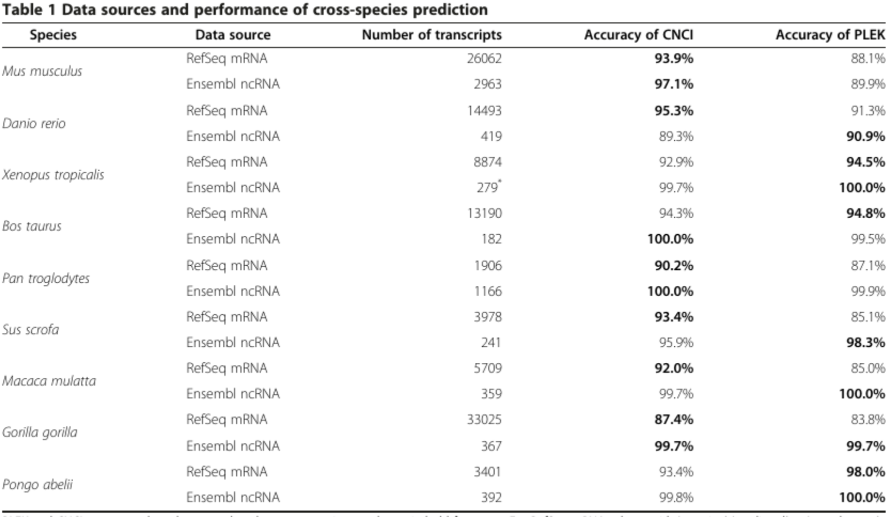


Table 1 Data sources and performance of cross-species prediction

Species	Data source	Number of transcripts	Accuracy of CNCI	Accuracy of PLEK
Mus musculus	RefSeq mRNA	26062	**93.9%**	88.1%
	Ensembl ncRNA	2963	**97.1%**	89.9%
Danio rerio	RefSeq mRNA	14493	**95.3%**	91.3%
	Ensembl ncRNA	419	89.3%	**90.9%**
Xenopus tropicalis	RefSeq mRNA	8874	92.9%	**94.5%**
	Ensembl ncRNA	279*	99.7%	**100.0%**
Bos taurus	RefSeq mRNA	13190	94.3%	**94.8%**
	Ensembl ncRNA	182	**100.0%**	99.5%
Pan troglodytes	RefSeq mRNA	1906	**90.2%**	87.1%
	Ensembl ncRNA	1166	**100.0%**	99.9%
Sus scrofa	RefSeq mRNA	3978	**93.4%**	85.1%
	Ensembl ncRNA	241	95.9%	**98.3%**
Macaca mulatta	RefSeq mRNA	5709	**92.0%**	85.0%
	Ensembl ncRNA	359	99.7%	**100.0%**
Gorilla gorilla	RefSeq mRNA	33025	**87.4%**	83.8%
	Ensembl ncRNA	367	**99.7%**	**99.7%**
Pongo abelii	RefSeq mRNA	3401	93.4%	**98.0%**
	Ensembl ncRNA	392	99.8%	**100.0%**

Figure: Data sources and performance of cross-species prediction

Robustness to indel sequencing errors

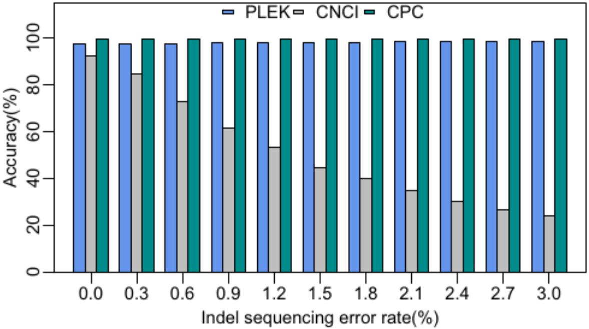
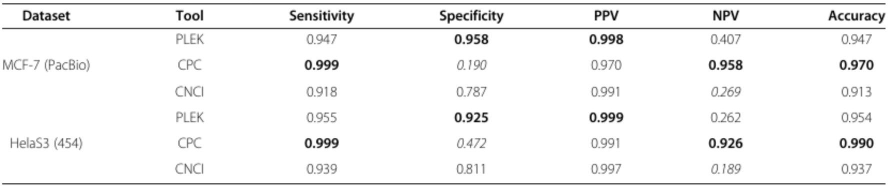


Figure: Comparison of robustness towards indel sequencing errors.

Robustness to indel sequencing errors



Dataset	Tool	Sensitivity	Specificity	PPV	NPV	Accuracy
MCF-7 (PacBio)	PLEK	0.947	**0.958**	**0.998**	0.407	0.947
	CPC	**0.999**	*0.190*	0.970	**0.958**	**0.970**
	CNCI	0.918	0.787	0.991	*0.269*	0.913
HelaS3 (454)	PLEK	0.955	**0.925**	**0.999**	0.262	0.954
	CPC	**0.999**	*0.472*	0.991	**0.926**	**0.990**
	CNCI	0.939	0.811	0.997	*0.189*	0.937

Figure: Performances on transcripts derived from PacBio and 454

Performance comparison on mouse datasets

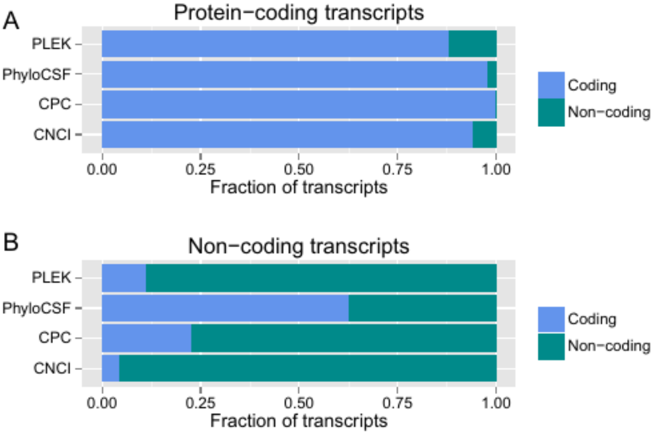
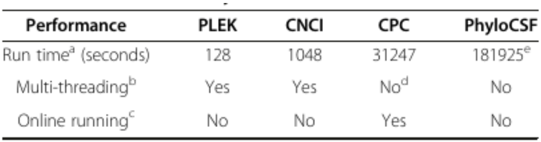


Figure: Results of PLEK, CPC, CNCI and PhyloCSF on mouse datasets.

Computational performance



Performance	PLEK	CNCI	CPC	PhyloCSF
Run time[a] (seconds)	128	1048	31247	181925[e]
Multi-threading[b]	Yes	Yes	No[d]	No
Online running[c]	No	No	Yes	No

Figure: Comparison of computational performances of PLEK, CNCI, CPC and PhyloCSF

Discussion

Prediction accuracy increases with the increasing k; however, this is accompanied by an increasing computation load.

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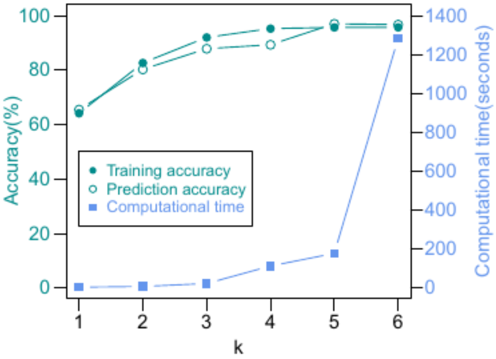

Figure: Performance comparison of various ranges of k.

Conclusion

PLEK is a useful tool for distinguishing protein-coding and non-coding sequences from high-throughput sequencing data of many species without reference genomes.

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