Supplementary Information

PlasmidHunter: Accurate and fast prediction of plasmid sequences using gene content profile and machine learning

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Supplementary Table 1. Summary of the confirmed chromosomes and plasmids for the modeling based on the annotation from NCBI database and the sizes. The sequences determined as chromosome or plasmid were included in the subsequent analaysis.

Annotation	Size	Determined as	Count
chromosome	> 900 Kbp	chromosome	25651
chromosome	<= 900 Kbp	not sure	713
plasmid	< 600 Kbp	plasmid	26136
plasmid	>= 600 Kbp	not sure	598
extrachrom		not sure	7
genomic		not sure	5

Supplementary Table 2. The evaluation of the performance of models predicting the location (chromosome or plasmid) of contigs. The evaluation was based on the model predictions on validation data using the package scikit-learn. RF = random forest; DT = decision tree; NB = naïve bayes; LR = logistic regression. Methods beginning with principal component analysis (PCA) means the modeling using PCA transformed data. Recall was calculated as TP/(TP+FN) and Precision was calculated as TP/(TP+FP), where TP is true positive, FN is false negative, and FP is false positive. F1 Score was calculated as 2*(Recall * Precision) / (Recall + Precision).

Methods	Accuracy	Balanced accuracy	Log loss	Recall	Precision	F score	ROC AUC
RF	70.7%	68.1%	0.60	36.5%	99.2%	0.53	0.87
DT	72.2%	69.9%	0.60	40.3%	98.4%	0.57	0.70
NB	***95.9%***	***95.9%***	***1.41***	***95.1%***	***95.9%***	***0.96***	***0.96***
LR	95.5%	95.3%	0.16	92.2%	97.9%	0.95	0.99
PCA-RF	89.1%	88.6%	0.29	82.4%	93.1%	0.87	0.95
PCA-DT	84.9%	84.6%	3.93	80.4%	85.9%	0.83	0.86
PCA-NB	73.7%	71.6%	8.68	45.7%	94.1%	0.62	0.84
PCA-LR	78.2%	76.4%	0.45	54.3%	96.8%	0.70	0.90
PCA-KNN	87.1%	86.8%	0.99	82.6%	88.6%	0.85	0.93

Supplementary Table 3. The statistics of the benchmark data. The contigs of specified lengths were randomly simulated from 3,000 plasmids and 3,000 chromosomes that were not included in the modeling. Each data set has equal number of plasmid and chromosome sequences.

Bechmark data ID	Contig length (Kbp)	Number of sequences *	Total length (Mbp)
1	5	6000	30
2	10	5230	52.3
3	20	4746	94.92
4	50	3520	176
5	100	1996	199.6

* The total number of sequences were less than 6000 in some data sets because some plasmids were shorter than the required lengths and were thus not included.

Supplementary Table 4. The evaluation of PlasmidHunter's performance on the benchmark data with different lengths. The prediction was run on a computer with eight processors (AMD EPYC 7551, 1.2 GHz) assigned to the task. The evaluation was conducted using the package scikit-learn. Recall was calculated as TP/(TP+FN) and Precision was calculated as TP/(TP+FP), where TP is true positive, FN is false negative, and FP is false positive. F1 Score was calculated as 2*(Recall * Precision) / (Recall + Precision).

Contig length (Kbp)	Accuracy	Balanced accuracy	Log loss	Recall	Precision	F score	ROC AUC	Time used (min)
5	87.70%	87.80%	4.257	93.80%	83.30%	88.20%	87.80%	1.9
10	91.40%	91.40%	2.983	94.10%	89.10%	91.50%	91.40%	2.4
20	94.20%	94.20%	1.993	94.20%	94.30%	94.20%	94.20%	3.6
50	96.40%	96.40%	1.257	95.20%	97.40%	96.30%	96.40%	6.5
100	96.70%	96.70%	1.142	94.20%	99.20%	96.60%	96.70%	7.7

Supplementary Table 5. The evaluation of Deeplasmid (version of Feb. 10, 2022) performance on the benchmark data with different lengths. The prediction was run on a different computer with eight processors (Intel Core i7-10510U, 1.8 GHz) because the Deeplasmid cannot be limited to use only eight processors in the other computer with 128 processors. Note that CPU mode rather than GPU mode of Deeplasmid was used in this running. The evaluation was conducted using the package scikit-learn. Recall was calculated as TP/(TP+FN) and Precision was calculated as TP/(TP+FP), where TP is true positive, FN is false negative, and FP is false positive. F1 Score was calculated as 2*(Recall * Precision) / (Recall + Precision).

Contig length (Kbp)	Accuracy	Balanced accuracy	Log loss	Recall	Precision	F score	ROC AUC	Time used (min)
5	65.8%	65.8%	1.340	32.6%	96.9%	48.8%	86.2%	742
10	66.6%	66.6%	1.901	34.2%	97.1%	50.6%	88.8%	758
20	73.8%	73.8%	1.971	48.9%	97.4%	65.1%	92.3%	773
50	86.8%	86.8%	1.278	74.8%	98.4%	85.0%	95.6%	776
100	92.5%	92.5%	0.623	86.4%	98.5%	92.0%	97.8%	543

Supplementary Table 6. The evaluation of PlasmidVerify (version of April 30, 2020) performance on the benchmark data with different lengths. The prediction was run on a computer with eight processors (AMD EPYC 7551, 1.2 GHz) assigned to the task. The evaluation was conducted using the package scikit-learn. The log loss and ROC AUC were not calculated because PlasmidVerify outputs likelihood ratios rather than probabilities of predictions. Recall was calculated as TP/(TP+FN) and Precision was calculated as TP/(TP+FP), where TP is true positive, FN is false negative, and FP is false positive. F1 Score was calculated as 2*(Recall * Precision) / (Recall + Precision).

Contig length (Kbp)	Accuracy	Balanced accuracy	Recall	Precision	F score	Time used (min)
5	78.42%	78.42%	58.43%	97.33%	73.03%	41.8
10	84.44%	84.44%	71.09%	96.97%	82.04%	63.2
20	89.78%	89.78%	82.98%	96.05%	89.03%	114.3
50	93.98%	93.98%	90.57%	97.20%	93.76%	213.6
100	94.94%	94.94%	91.68%	98.07%	94.77%	239.5

Supplementary Table 7. The evaluation of PlasFlow (version 1.1.0, August 15, 2018) performance on the benchmark data with different lengths. The prediction was run on a computer with one processors (AMD EPYC 7551, 1.2 GHz) assigned to the task, because PlasmidFlow does not have multiple processing mode. Threshold of 0.5 was used for classification to eliminate unclassified prediction. The evaluation was conducted using the package scikit-learn. The log loss and ROC AUC were not calculated because PlasFlow does not output probability of predictions plasmid or chromosome. Recall was calculated as TP/(TP+FN) and Precision was calculated as TP/(TP+FP), where TP is true positive, FN is false negative, and FP is false positive. F1 Score was calculated as 2*(Recall * Precision) / (Recall + Precision).

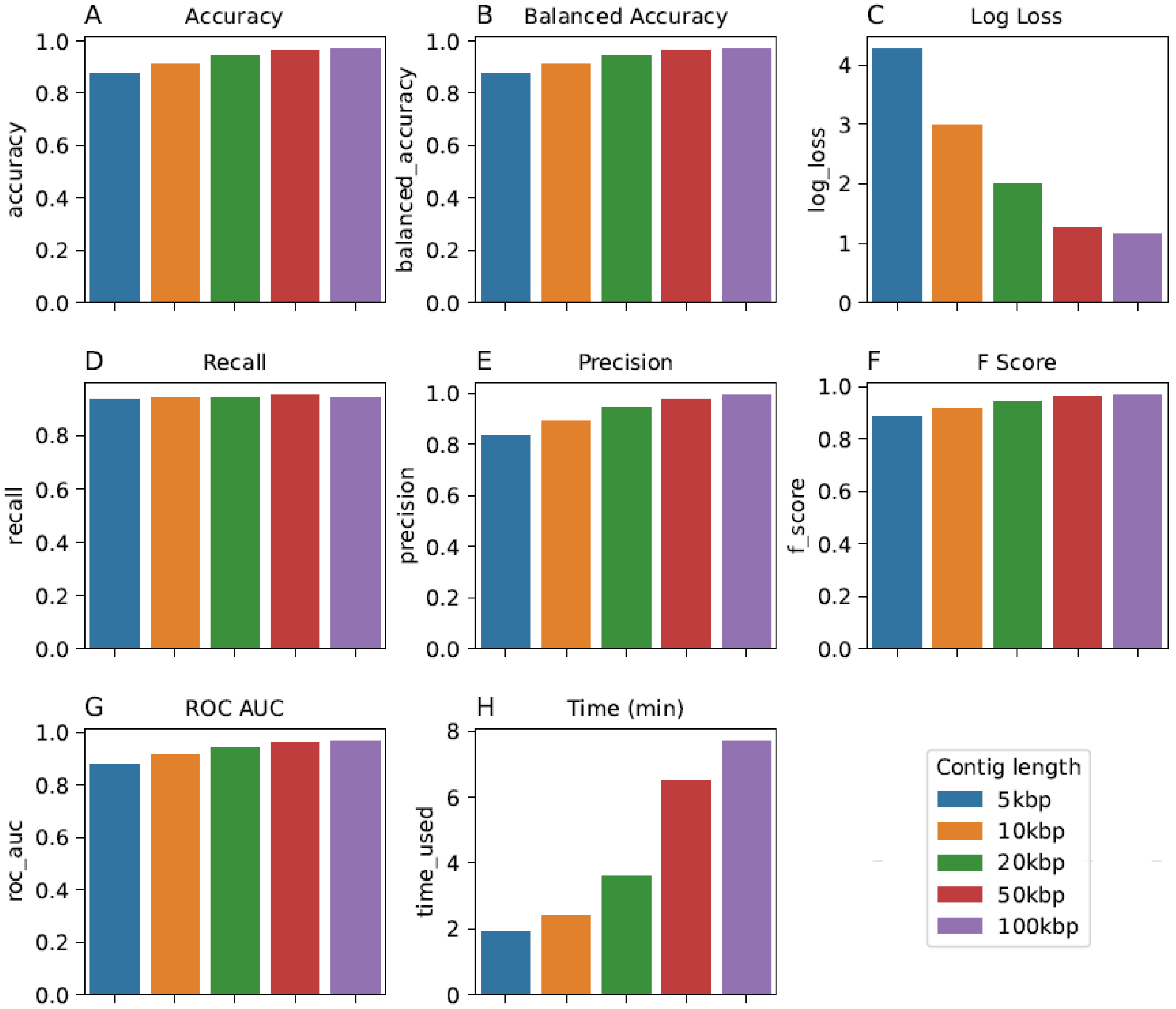
Contig length (Kbp)	Accuracy	Balanced accuracy	Recall	Precision	F score	Time used (min)
5	83.23%	83.23%	88.40%	80.12%	84.06%	0.5
10	87.97%	87.97%	89.94%	86.53%	88.21%	0.6
20	90.67%	90.67%	89.55%	91.59%	90.56%	0.7
50	91.76%	91.76%	88.98%	94.22%	91.53%	0.8
100	92.84%	92.84%	89.68%	95.72%	92.60%	0.8

Supplementary Table 8. The evaluation of PlasForest (version of October 14, 2021) performance on the benchmark data with different lengths. The prediction was run on a computer with eight processors (AMD EPYC 7551, 1.2 GHz) assigned to the task. The evaluation was conducted using the package scikit-learn. The log loss and ROC AUC were not calculated because PlasForest does not output probability of prediction. Recall was calculated as TP/(TP+FN) and Precision was calculated as TP/(TP+FP), where TP is true positive, FN is false negative, and FP is false positive. F1 Score was calculated as 2*(Recall * Precision) / (Recall + Precision).

Contig length (Kbp)	Accuracy	Balanced accuracy	Recall	Precision	F score	Time used (min)
5	80.45%	80.45%	61.90%	98.41%	76.00%	9
10	81.05%	81.05%	63.14%	98.39%	76.92%	12.8
20	81.42%	81.42%	63.42%	99.08%	77.34%	19.9
50	80.45%	80.45%	61.14%	99.63%	75.77%	27.5
100	77.35%	77.35%	55.11%	99.28%	70.88%	22.5

Supplementary Table 9. The evaluation of PlasClass (version 0.1.1, November 1, 2021) performance on the benchmark data with different lengths. The prediction was run on a computer with eight processors (AMD EPYC 7551, 1.2 GHz) assigned to the task. The evaluation was conducted using the package scikit-learn. Recall was calculated as TP/(TP+FN) and Precision was calculated as TP/(TP+FP), where TP is true positive, FN is false negative, and FP is false positive. F1 Score was calculated as 2*(Recall * Precision) / (Recall + Precision).

Contig length (Kbp)	Accuracy	Balanced accuracy	Log loss	Recall	Precision	F score	ROC AUC	Time used (min)
5	82.37%	82.37%	0.42	83.90%	81.40%	82.63%	90.44%	0.5
10	88.09%	88.09%	0.30	88.18%	88.02%	88.10%	94.71%	0.6
20	91.15%	91.15%	0.23	91.15%	91.15%	91.15%	96.76%	1.1
50	93.92%	93.92%	0.18	94.66%	93.28%	93.97%	98.13%	1.8
100	94.39%	94.39%	0.68	91.98%	96.63%	94.25%	97.94%	2



Supplementary Figure 1. The visualized evaluation of PlasmidHunter's performance on the benchmark data with different lengths. The prediction was run on a computer with eight processors (AMD EPYC 7551, 1.2 GHz) assigned to the task. The evaluation was conducted using the package scikit-learn.