Supplementary Information: Tables and Figures

CRISPR-Cas-Docker: Web-based *in silico* docking and machine learning-based classification of crRNAs to Cas proteins

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Figure S1. The architecture of CRISPR-Cas-Docker. This diagram shows how users interact with the Server to make service requests and view results. The *Server* manages these interactions through the *Home* and *Result* interfaces. The *Worker* component is responsible for generating the actual results, with the *Server* and *Worker* exchanging data through Storage without direct communication. The black arrow represents the request of a user, while the red arrow shows the generation of results. The blue arrow indicates the user interaction with the results. CRISPR-Cas-Docker is implemented by making use of the following Python libraries or binaries;

Server: Flask, BioPython, Plotly, NumPy, Pandas

Workers: Scikit-learn, RNAfold, RoseTTAFold, AlphaFold, HDOCK.

*The source of the libraries and binaries can be found in the section on Data and Code Availability of the main manuscript.

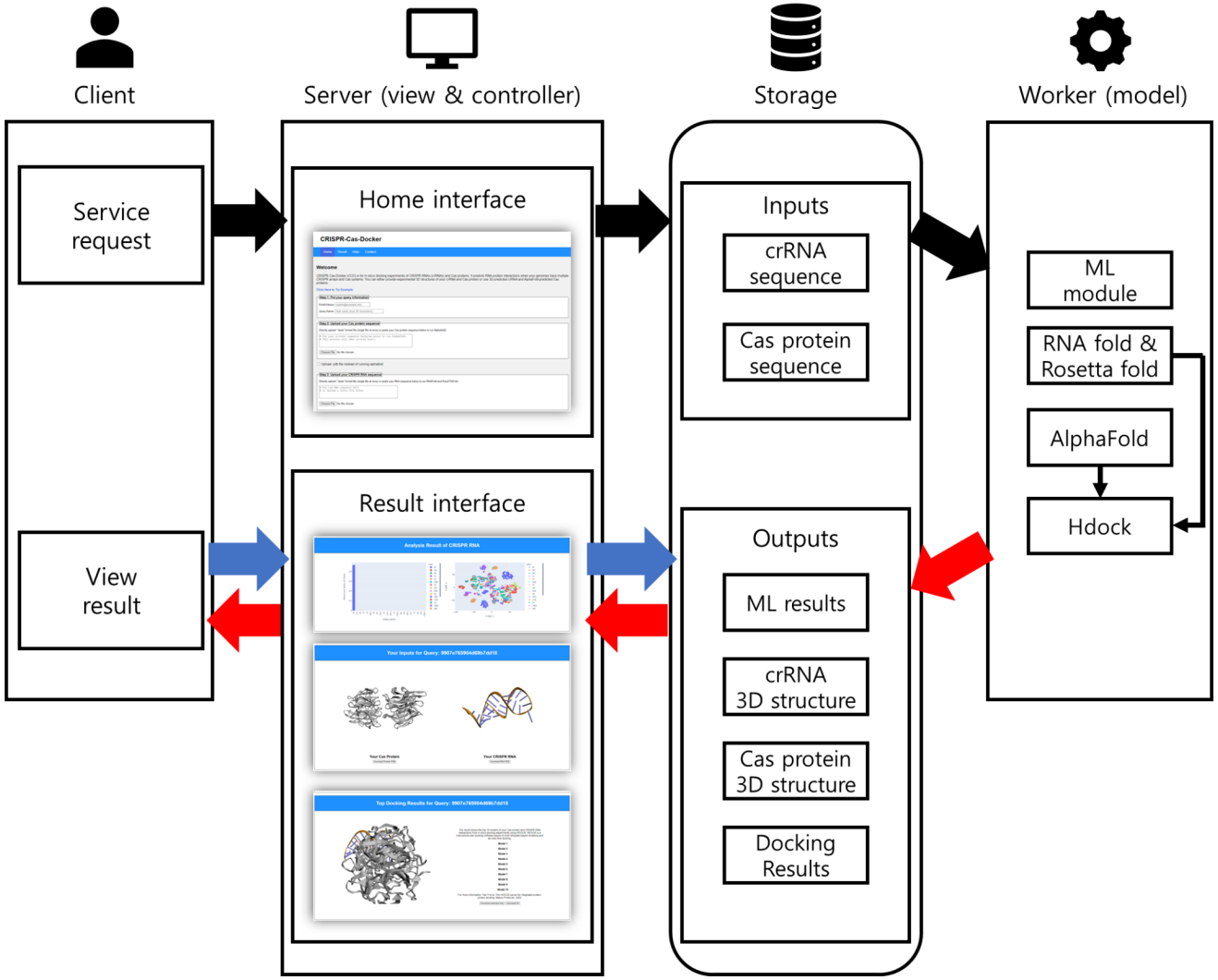


Figure S2. Averaged boxplot of CRISPR-Cas-Docker performance for Cas13 proteins. In particular, this boxplot shows that the average docking score is approximately -600 for all four Cas proteins, with no noticeable differences between them. However, there are some particularly low outliers for GTP-GTR, which may be indicative of docking performance very close to the ground truth. According to the HDOCK server, a lower docking score corresponds to a better docking model. (GTP: Ground Truth Cas Protein; GTR: Ground Truth crRNA; PP: Predicted Cas Protein (AlphaFold); PR: Predicted crRNA (RoseTTAFold)).

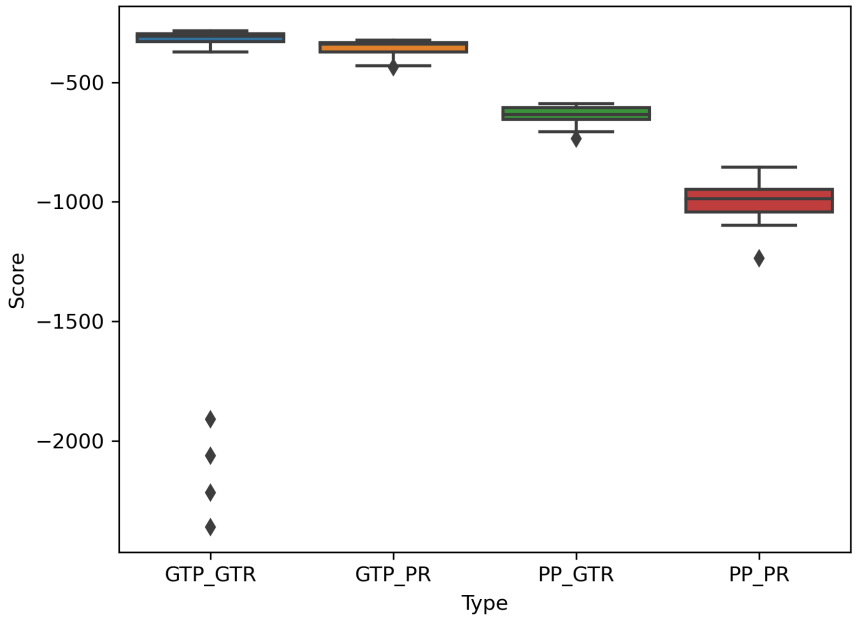


Figure S3. CRISPR repeat sequences labeled by the adjacent Cas system (±10,000 base pairs). As the created dataset has a class imbalance, we divided the CRISPR repeat sequences into four subsets based on their frequency of occurrence. This ensures that the KNN classification is not affected by the aforementioned class imbalance. The first subset, which is named Major, includes IE, IIC, IB, IC, IF, and IIIA, with each class containing more than 1,000 instances. Since the number of IE instances (6,862) is four or more times that of other types, 20% of the IE repeats were randomly sampled for training (1,372). The second subset, which is called Minor, includes IIIB, IA, IIA, and IIID, with each class having more than 300 instances and not belonging to Major. The third subset is named Tiny, which includes classes with less than 300 instances and with these classes not belonging to either Major or Minor. Lastly, the subset Undefined consists of CAS and IU which are Cas system types that are not complete and unidentified, respectively.

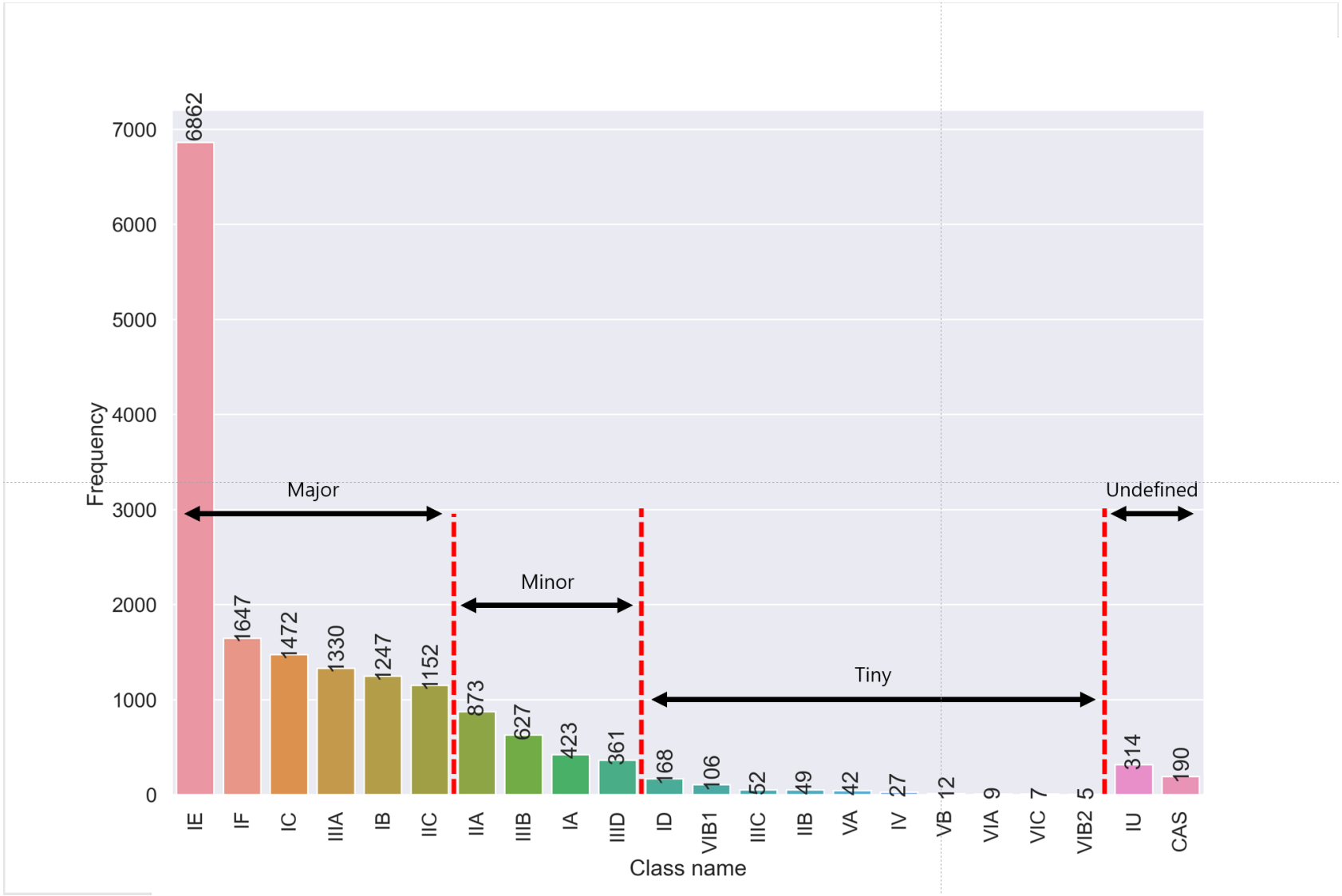


Figure S4. Distance distributions of CRISPR repeat sequences labeled with their adjacent Cas system. This histogram shows the distance of each CRISPR array to the adjacent Cas system in base pairs. It shows that most CRISPR arrays are around 100 base pairs away from their adjacent Cas system (2859), but there are some as far as 10,000 base pairs away.

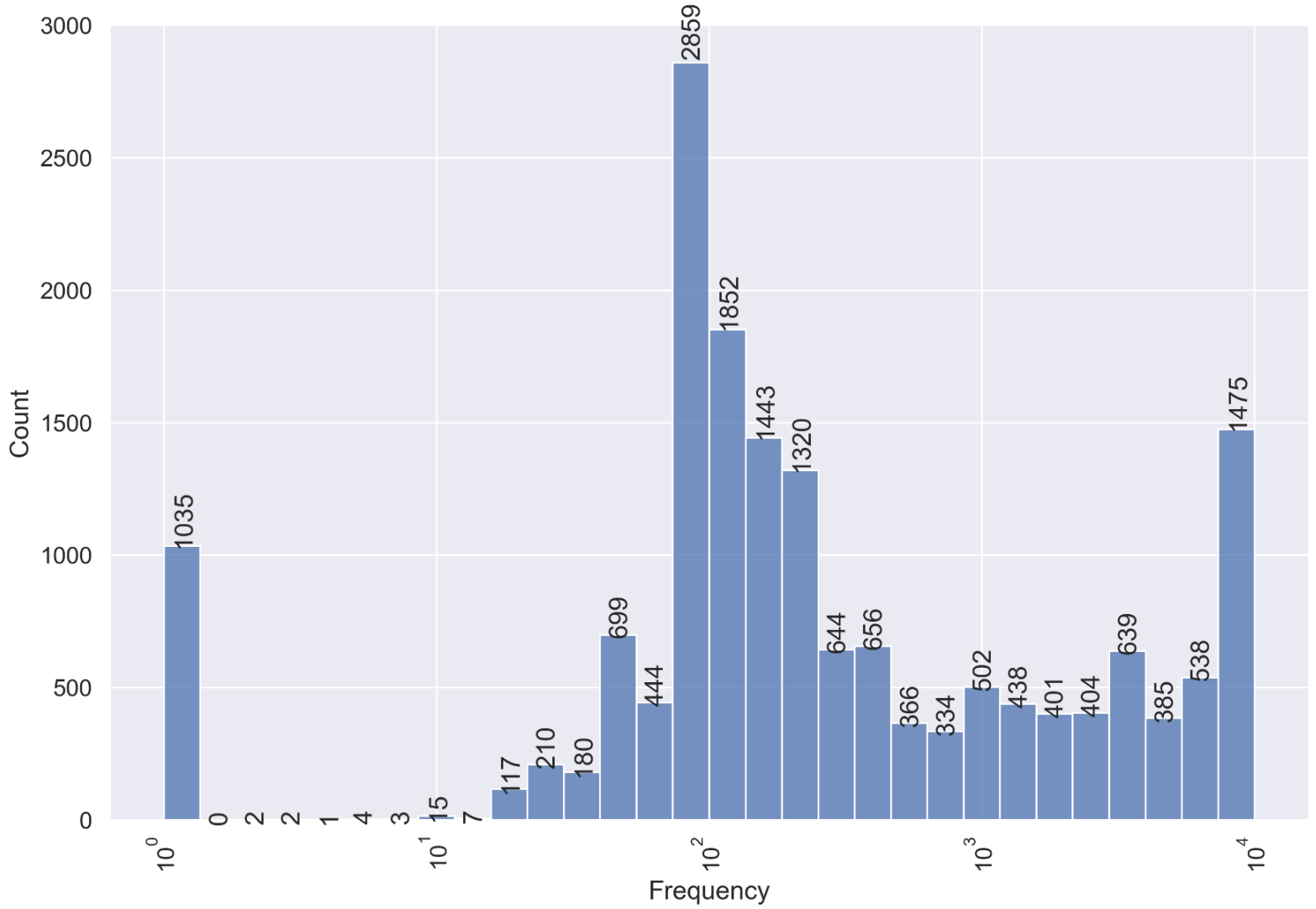


Figure S5. Length distribution of CRISPR repeat sequences labeled with their adjacent Cas system (±10,000 base pairs). The number next to the type of CRISPR repeats at the top of each histogram shows the average length (standard deviations), which indicates that the average length of CRISPR repeats varies by the associated Cas system.

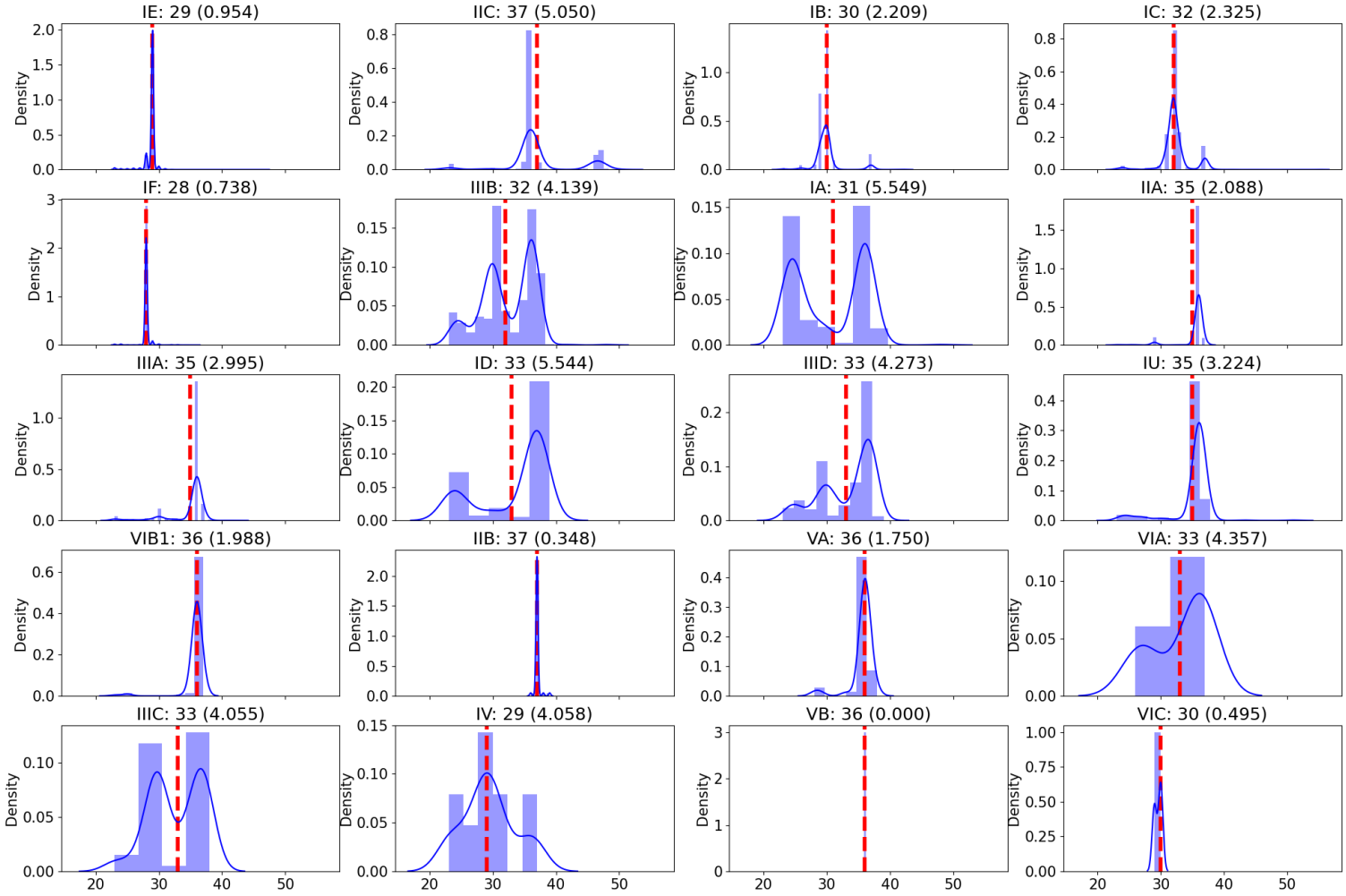


Figure S6. Two-dimensional CRISPR sequence atlas. The interactive version is available in CRISPR-Cas-Docker. We used t-SNE to show the Hamming distance between all pairs of sequences in a two-dimensional representation. Each dot represents a crRNA sequence, with the shape and color of the dot indicating the type of that particular crRNA sequence. According to the t-SNE method, closely located dots denote similar sequences. We pad the shorter sequence with padding characters in order to equalize their lengths when using the Hamming distance measure.

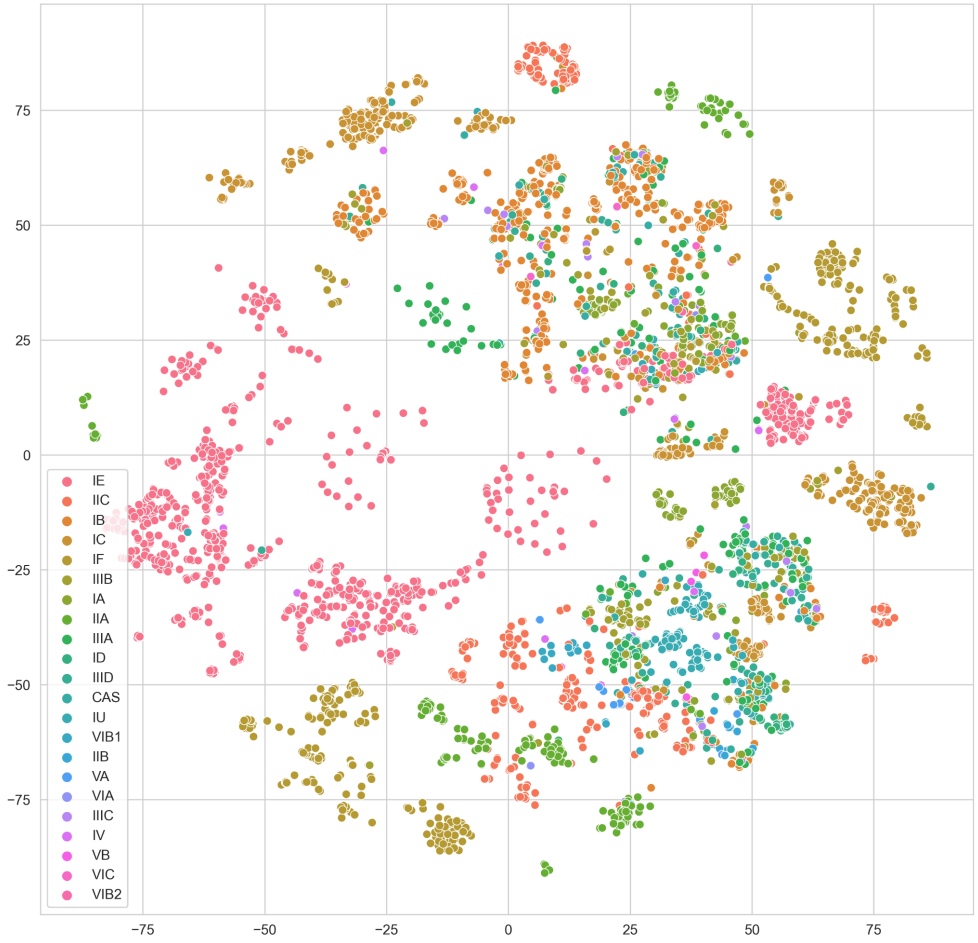
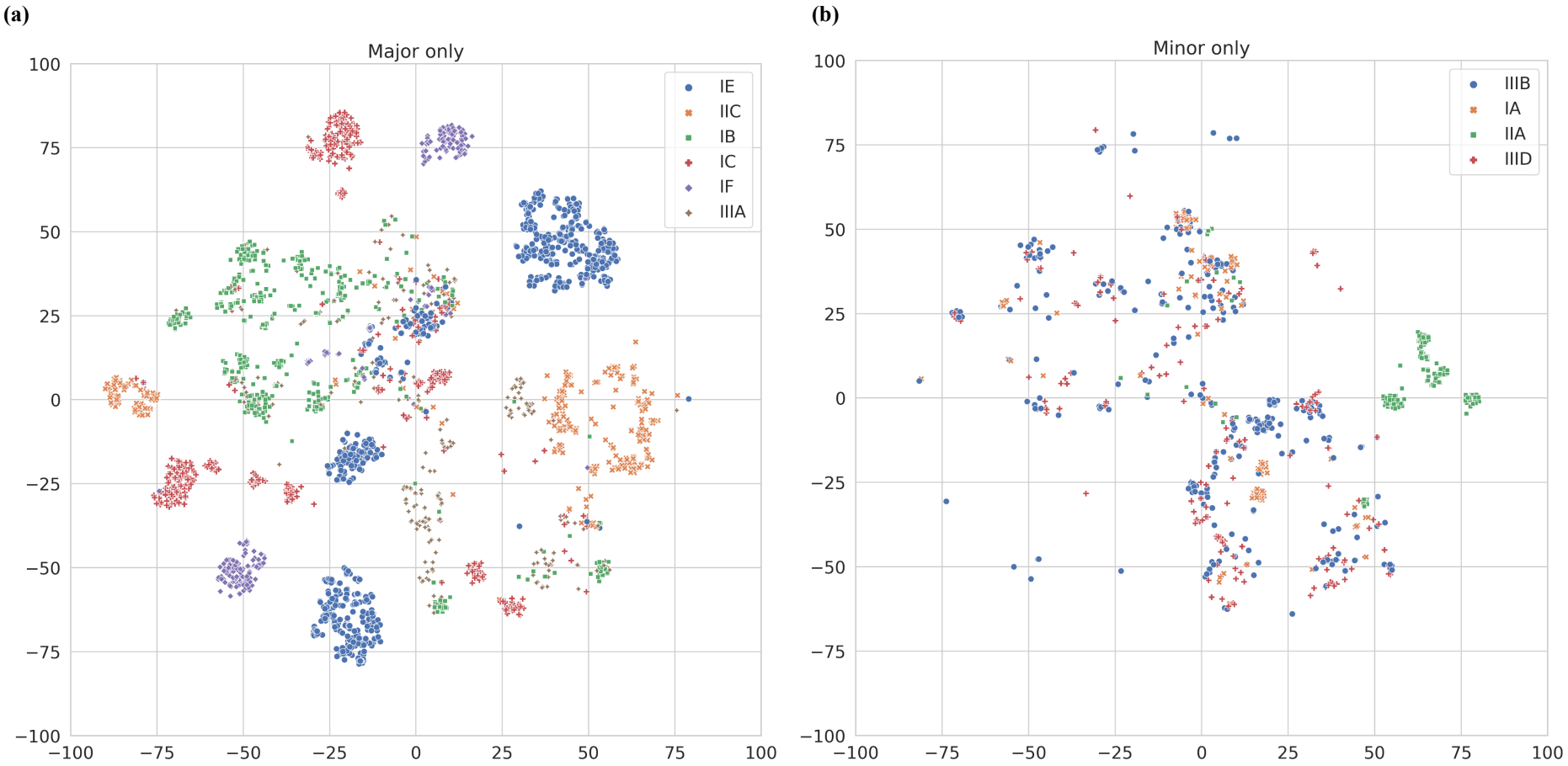
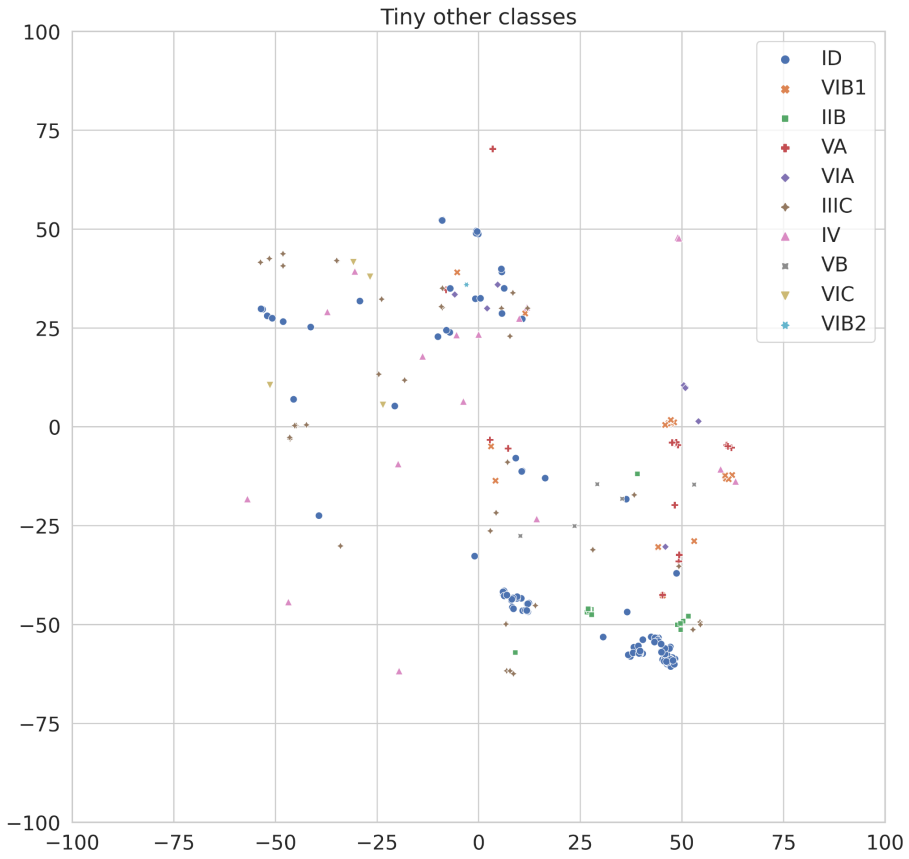


Figure S7. Two-dimensional CRISPR sequence atlas, separated by the data subsets. We divide the crRNA sequence data into (a) Major (more than 1,000 sequences), (b) Minor (more than 300 sequences), (c) Tiny (less than 300 sequences), and (d) Undefined (CAS and IU types). In the case of the Major subset, we found that the IE type has four clusters, and the cluster located near (0, 25) overlaps heavily with other types. In the case of the Minor subset, we found many overlapping points in most of the types, except for IIID. These overlapping points suggest that a single crRNA sequence may be labeled with multiple Cas system types.



(c)


Tiny other classes
ID
VIB1
IIB
VA
VIA
IIIC
IV
VB
VIC
VIB2
100
75
50
25
0
−25
−50
−75
−100
−100
−75
−50
−25
0
25
50
75
100

(d)

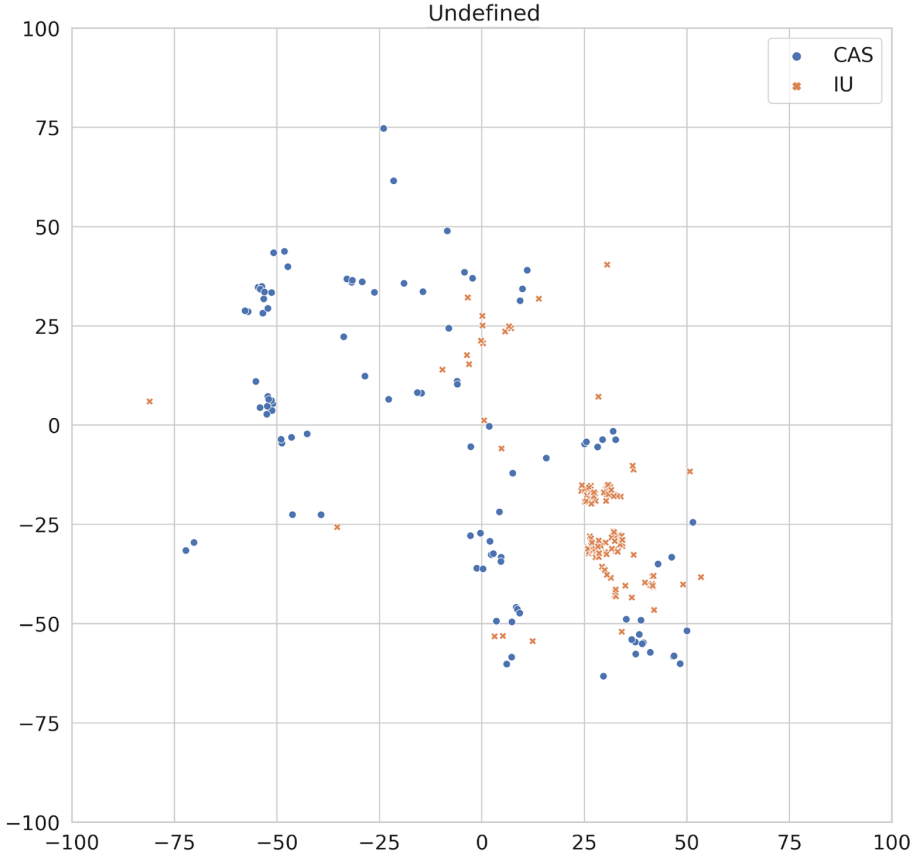

Undefined
CAS
IU
100
75
50
25
0
−25
−50
−75
−100
−100
−75
−50
−25
0
25
50
75
100

Figure S8. Confusion matrix of the classification result. The matrix is divided into four subsets for easier viewing. Except for the Tiny subset, which has a small number of test sets, IB had the highest misclassification rate in the Major subset. We observed that IB is frequently mistaken for IIIB. In the Minor subset, IIIB had the highest misclassification rate. This may be due to IIIB sharing many similar sequences with other CRISPR arrays. Overall, the KNN model performed well in correctly classifying the Major and Minor classes, with the aforementioned exceptions.

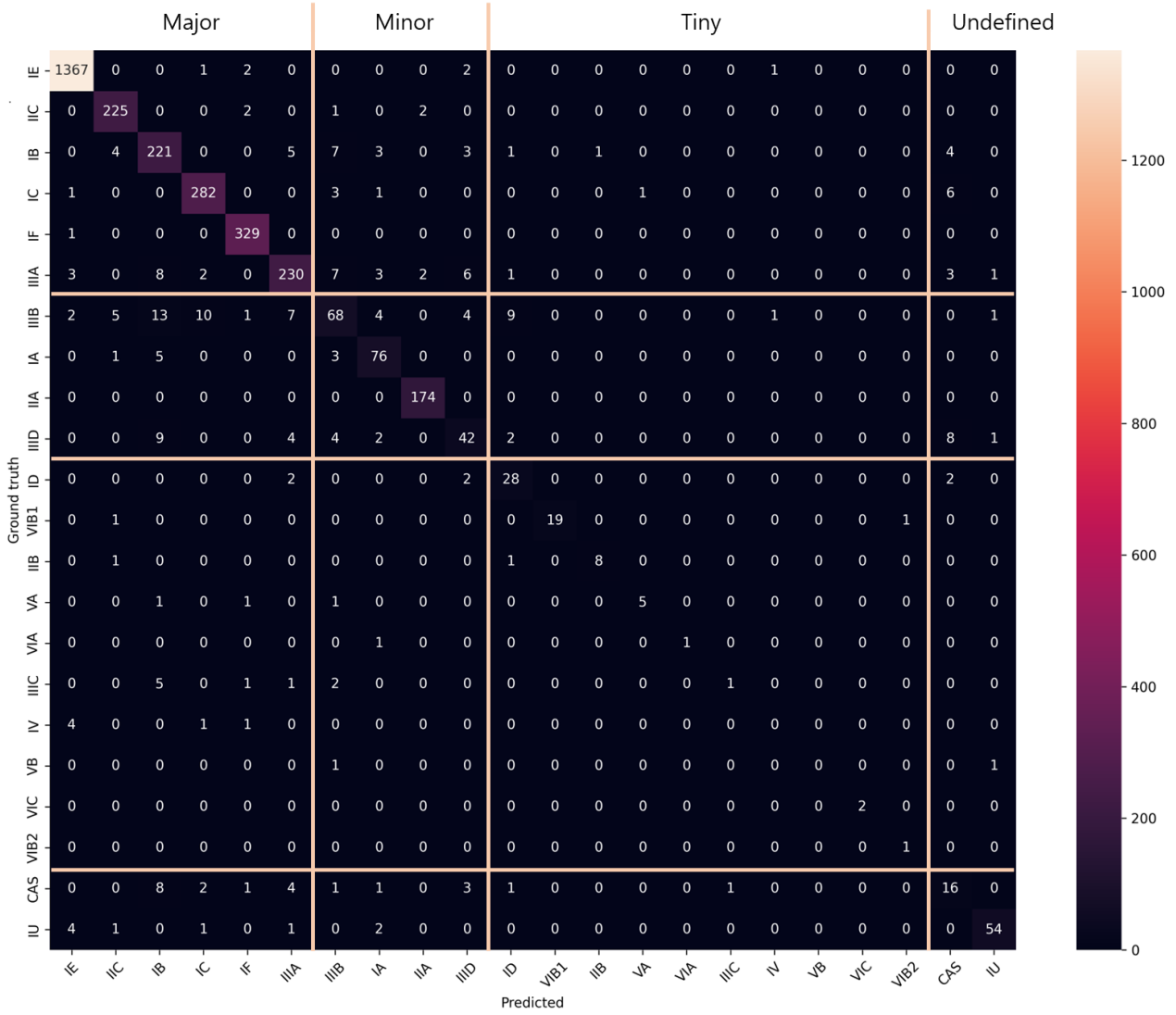


Table S1: TM-scores of AlphaFold-predicted Cas proteins.

TM-score (0,1] to measure the folding performance of AlphaFold.
0.0 < TM-score < 0.30, random structural similarity
0.5 < TM-score ≤ 1.00, high structural similarity (about the same fold)

RMSD to check the atom level structure difference.
RMSD < 2Å: two structures are the same structure.

TM-Score	5w1h	5w1i_AB	5w1i_CD	5wlh
With template	0.99390	0.99240	0.99077	0.99403
No template	0.80226	0.83607	0.81096	0.81735

RMSD	5w1h	5w1i_AB	5w1i_CD	5wlh
With template	1.18	1.23	1.31	1.31
No template	4.07	4.03	4.00	4.40

Table S2. Distribution of CRISPR array evidence level by type. The numbered suffix (1 to 4) indicates the evidence level, which is assigned based on the combined degree of similarity of repeats and spacers (Couvin et al. 2018). A higher evidence level indicates a higher chance that the sequence corresponds to a CRISPR array.

Cas with evidence level	Frequency	Percentage		Cas with evidence level	Frequency	Percentage
Cas-Type IE 1	143	0.021		CAS 1	18	0.095
Cas-Type IE 2	28	0.004		CAS 2	4	0.021
Cas-Type IE 3	37	0.005		CAS 3	5	0.026
Cas-Type IE 4	6654	0.970		CAS 4	163	0.858
Cas-Type IIC 1	47	0.041		Cas-Type IU 1	18	0.057
Cas-Type IIC 2	1	0.001		Cas-Type IU 2	1	0.003
Cas-Type IIC 3	16	0.014		Cas-Type IU 3	11	0.035
Cas-Type IIC 4	1088	0.944		Cas-Type IU 4	284	0.904
Cas-Type IB 1	68	0.055		Cas-Type VIB1 1	1	0.009
Cas-Type IB 2	8	0.006		Cas-Type VIB1 2	2	0.019
Cas-Type IB 3	9	0.007		Cas-Type VIB1 3	1	0.009
Cas-Type IB 4	1162	0.932		Cas-Type VIB1 4	102	0.962
Cas-Type IC 1	30	0.020		Cas-Type IIB 1	0	0.000
Cas-Type IC 2	23	0.016		Cas-Type IIB 2	1	0.020
Cas-Type IC 3	20	0.014		Cas-Type IIB 3	1	0.020
Cas-Type IC 4	1399	0.950		Cas-Type IIB 4	47	0.959
Cas-Type IF 1	15	0.009		Cas-Type VA 1	1	0.024
Cas-Type IF 2	0	0.000		Cas-Type VA 2	0	0.000
Cas-Type IF 3	7	0.004		Cas-Type VA 3	1	0.024
Cas-Type IF 4	1625	0.987		Cas-Type VA 4	40	0.952
Cas-Type IIIB 1	49	0.078		Cas-Type VIA 1	3	0.333
Cas-Type IIIB 2	0	0.000		Cas-Type VIA 2	0	0.000
Cas-Type IIIB 3	20	0.032		Cas-Type VIA 3	2	0.222
Cas-Type IIIB 4	558	0.890		Cas-Type VIA 4	4	0.444
Cas-Type IA 1	34	0.080		Cas-Type IIIC 1	11	0.212
Cas-Type IA 2	1	0.002		Cas-Type IIIC 2	0	0.000
Cas-Type IA 3	11	0.026		Cas-Type IIIC 3	0	0.000
Cas-Type IA 4	377	0.891		Cas-Type IIIC 4	41	0.788
Cas-Type IIA 1	4	0.005		Cas-Type IV 1	7	0.259
Cas-Type IIA 2	0	0.000		Cas-Type IV 2	4	0.148
Cas-Type IIA 3	14	0.016		Cas-Type IV 3	1	0.037
Cas-Type IIA 4	855	0.979		Cas-Type IV 4	15	0.556
Cas-Type IIIA 1	47	0.035		Cas-Type VB 1	0	0.000
Cas-Type IIIA 2	21	0.016		Cas-Type VB 2	0	0.000
Cas-Type IIIA 3	23	0.017		Cas-Type VB 3	0	0.000
Cas-Type IIIA 4	1239	0.932		Cas-Type VB 4	12	1.000
Cas-Type ID 1	19	0.113		Cas-Type VIC 1	5	0.714
Cas-Type ID 2	0	0.000		Cas-Type VIC 2	0	0.000
Cas-Type ID 3	1	0.006		Cas-Type VIC 3	0	0.000
Cas-Type ID 4	148	0.881		Cas-Type VIC 4	2	0.286
Cas-Type IIID 1	30	0.083		Cas-Type VIB2 1	0	0.000
Cas-Type IIID 2	2	0.006		Cas-Type VIB2 2	0	0.000
Cas-Type IIID 3	9	0.025		Cas-Type VIB2 3	1	0.200
Cas-Type IIID 4	320	0.886		Cas-Type VIB2 4	4	0.800

Table S3. Performance of the machine learning-based classification module in CRISPR-Cas-Docker. We used K-Nearest Neighbors (K=1) after experimenting with five different K values (1,3,5,7,9) with Hamming distance for the model. The dataset consisted of 16,972 crRNA sequences, with 80% of the data used for training and 20% for testing. The Support column indicates the number of actual instances of the Type in the test set. We pad the shorter sequence with padding characters in order to equalize their lengths when using the Hamming distance measure.

Hamming nearest neighbor classification					
Amount	Type	Precicion	Recall	F1score	Support
Major >1,000	IE	0.990	1.000	0.990	1373
	IIC	0.950	0.980	0.960	230
	IB	0.820	0.890	0.850	249
	IC	0.940	0.960	0.950	294
	IF	0.970	1.000	0.990	330
	IIIA	0.910	0.860	0.880	266
Minor >300	IIIB	0.690	0.540	0.610	125
	IA	0.820	0.890	0.850	85
	IIA	0.980	1.000	0.990	174
	IIID	0.680	0.580	0.630	72
Tiny <300	ID	0.650	0.820	0.730	34
	VIB1	1.000	0.900	0.950	21
	IIB	0.890	0.800	0.840	10
	VA	0.830	0.620	0.710	8
	VIA	1.000	0.500	0.670	2
	IIIC	0.500	0.100	0.170	10
	IV	0.000	0.000	0.000	6
	VB	0.000	0.000	0.000	2
	VIC	1.000	1.000	1.000	2
	VIB2	0.500	1.000	0.670	1
Undefined	CAS	0.410	0.420	0.420	38
	IU	0.930	0.860	0.890	63
Macro avg		0.750	0.720	0.720	3395
Weighted avg		0.920	0.930	0.920	3395
Accuracy		0.930			3395