Recognition of CRISPR/Cas9 off-target sites through ensemble learning of uneven mismatch distributions

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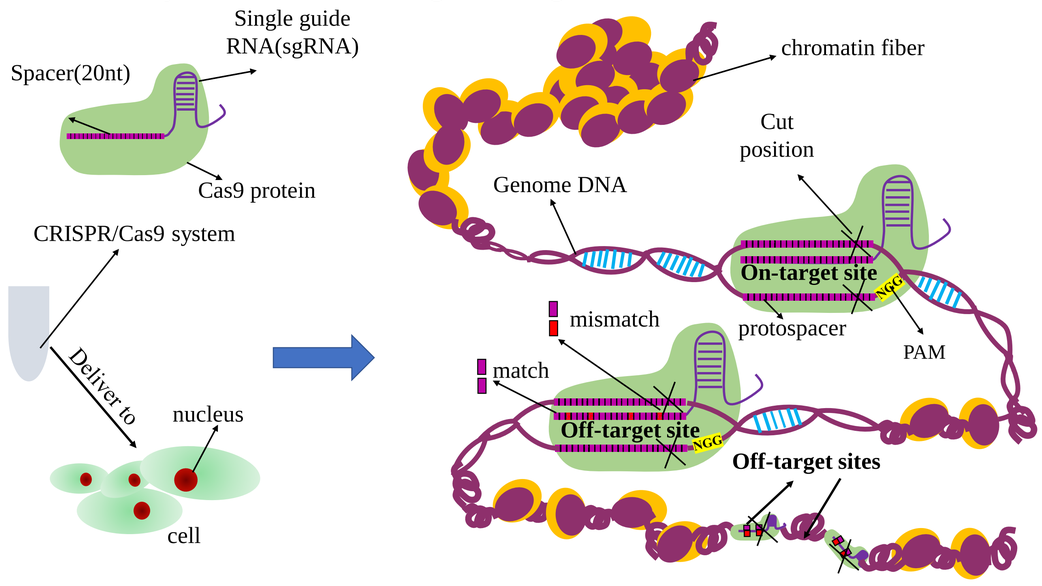
ABSTRACT

CRISPR/Cas9 is driving a broad range of innovative applications from basic biology to biotechnology and medicine. One of its current issues is the effect of off-target editing that should be critically resolved and should be completely avoided in the ideal use of this system. Here, we developed an ensemble learning method to detect the off-target sites of an sgRNA from its thousands of genome-wide candidates. Nucleotide mismatches between on-target and off-target sites have been studied recently. We confirm that there exist strong mismatch enrichment and preferences at the 5'-end close regions of the off-target sequences. Comparing with the on-target sites, sequences of no editing sites can be also characterized by GC composition changes and position-specific mismatch binary features. Under this novel space of features, an ensemble strategy was applied to train a prediction model. The model achieved a mean score 0.99 of Area Under Receiver Operating Characteristic curve (AUROC) and a mean score 0.45 of Area Under Precision-Recall curve (AUPRC) in cross-validations on big data sets, outperforming state-of-the-art methods in various test scenarios. Our predicted off-target sites also correspond well to those detected by high-throughput sequencing techniques. Especially, two case studies for selecting sgRNAs to cure hearing loss and retinal degeneration partly prove the effectiveness of our method.

BACKGROUND

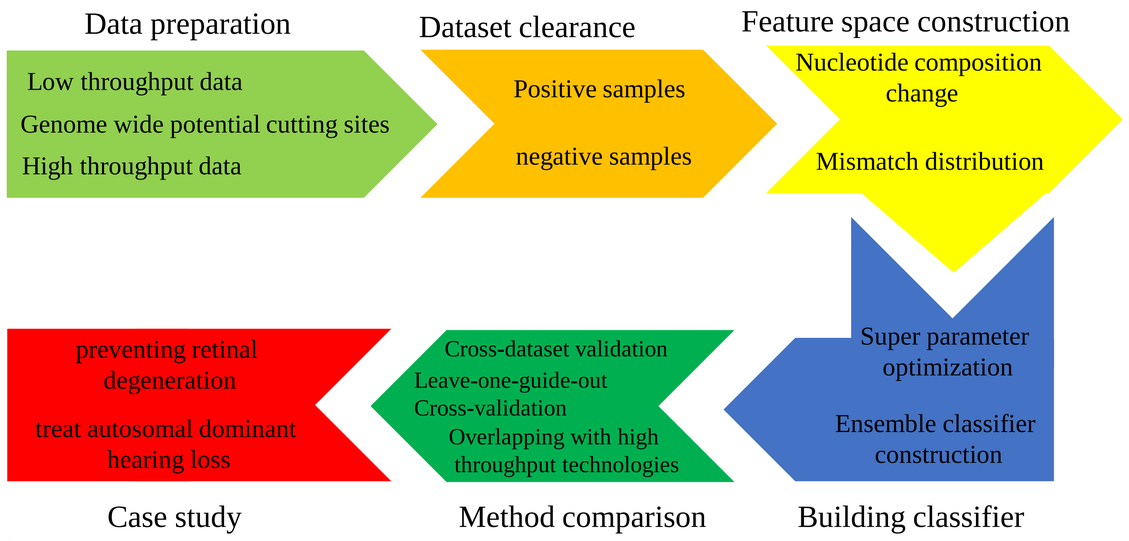
❑ CRISPR/Cas9 System and On/Off-target Editing



- **CRISPR/Cas9 complex**:
  part 1: a single-guide RNA(sgRNA) -- find target region (rule: 3nt PAM+20nt spacer, mismatches and indels can be tolerated); ***part 2***: a Cas9 protein -- cut the DNA.
- **On-target site**: the expected cutting region. **Off-target site**: the unintended cutting region.

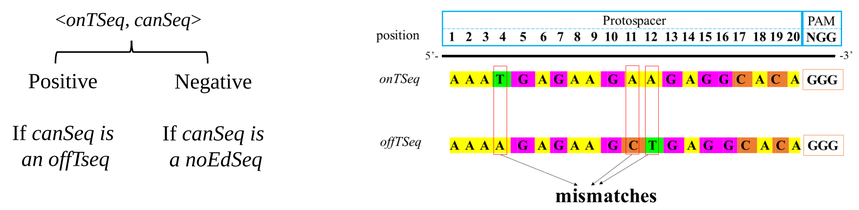
METHODOLOGY

❑ Flowchart



❑ Dataset

❖ Sequence pair (sample)



Positive Sets:

- D_{+}^{low}: Low throughput technologies (PCR) (215 positive samples involving 29 sgRNAs)
- D_{+}^{high}: High throughput technologies (NGS)(527 positive samples involving 11 sgRNAs)

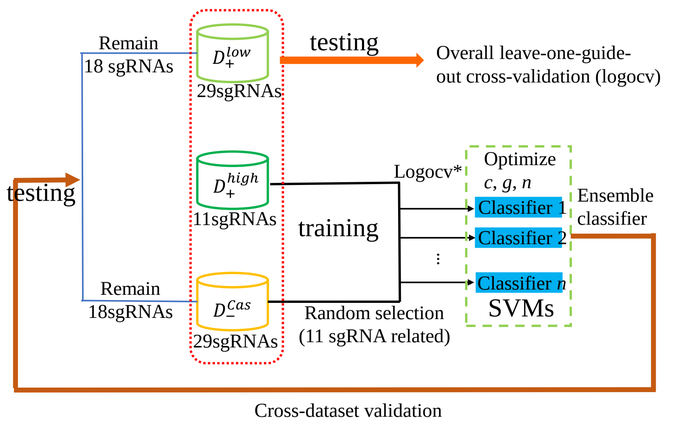
Negative Set:

- D_{-}^{Cas}: Cas-OFFinder tool (408260 negative samples involving 29 sgRNAs)

❑ Feature

- Nucleotide composition change features: <GC count change>, <GC percent change>, <GC skew change>, <AT skew change>, <Change of ratio of GC skew and AT skew>
- Position-specific binary mismatch features: <mismatch binary vector>

❑ Ensemble Learning Scheme



Optimization:

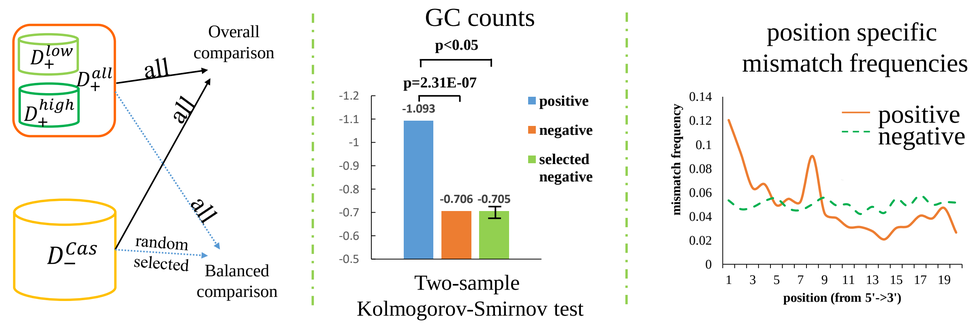
- ***Objective***: optimize super parameters
- ***Method***: leave-one-guide-out cross-validation (logocv), best AUROC
- ***Dataset***: D_{+}^{high} and D_{-}^{Cas} (11 sgRNAs, 11 folds)

Evaluation and comparison:

- ***Objective***: evaluate the classifier and compare with state-of-the-art methods
- ***Method***: Cross-dataset validation (cdv) and overall logocv, AUROC and AUPRC
- ***Dataset***: cdv—all samples exclude above Dataset (18sgRNAs); overall logocv—all samples (29 sgRNAs)

RESULTS

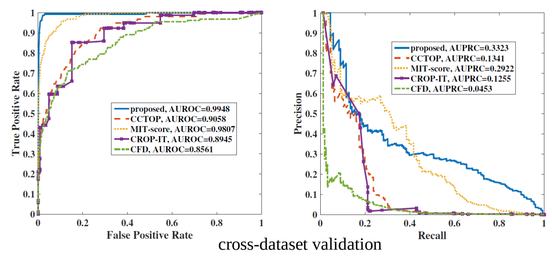
❑ Important sample difference



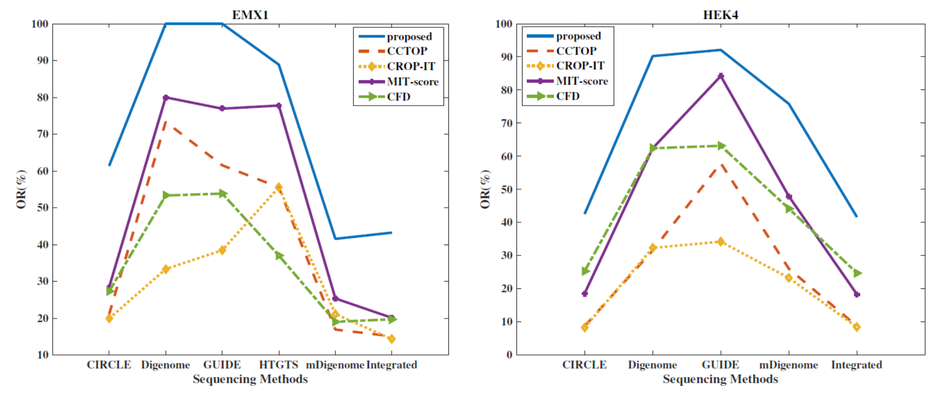
❑ Performance Comparison

- Performance Comparison in cross-dataset validation and leave-one-guide-out cross validation

Methods	cross-dataset validation		logocv[a]	
	AUROC	AUPRC	AUROC	AUPRC
proposed	0.9948	0.3323	0.9926	0.4571
CCTOP	0.9058	0.1341	0.9021	0.1407
MIT-score	0.9807	0.2922	0.9783	0.2960
CROP-IT	0.8945	0.1255	0.9160	0.1086
CFD	0.8561	0.0453	0.8835	0.0844



- Comparison of the off-target sites detected by the computational methods and those by the high-throughput sequencing methods



Overlap rate (OR): $\mathrm{OR}\% = \frac{overlapped\ number\ (com \cap wet\text{-}lab)}{number\ of\ wet\text{-}lab\ technique\ detections\ (wet\text{-}lab)} \times 100\%$

❑ Case Study: Choose optimal sgRNA for curing diseases

- Case One: editing Nrl gene to treat retinal degeneration
- Case Two: editing TMC1 gene to treat human autosomal dominant hearing loss gene

Table 3. The ranks of the sgRNAs by considering both of their cutting efficiencies and off-target potentials.

	sgRNA	literature			proposed			CRISPR Design			sgRNA Designer		
		Indel(%)	efficiency rank	final rank	ot number	ot rank	final rank	ot number	ot rank	final rank	ot number	ot rank	finak rank
Case one	NT1	21.9	4	-	264	5	5	101	2	2	-	1	3
	NT2	22.7	2	**1**	83	1	**1**	69	1	1	-	2	**1**
	NT3	22.5	3	-	139	4	3	159	4	4	-	5	4
	NT4	23.2	1	-	119	3	2	146	5	5	-	3	**1**
	NT5	18.3	5	-	95	2	3	115	3	3	-	4	5
Case two	Tmc1-mut1	4.1	2	-	613	3	3	337	3	3	-	1	**1**
	Tmc1-mut2	0.74	3	-	183	1	2	318	2	2	-	3	2
	Tmc1-mut3	10	1	**1**	247	2	**1**	197	1	**1**	-	2	3

- **ot number**: predicted off-target sites numbers
- **efficiency rank**: rank sgRNA by on target cutting efficiency; **ot rank**: rank sgRNA by off-target site number; **final rank**: (efficiency rank + ot rank)/2, final selection guidance

❑ Conclusion

Contribution:

- Turn the off-target site detection problem into a binary classification issue
- Define the sample as a sequence pair and take nucleotide composition changes and mismatch distribution properties as novel features
- Propose an ensemble learning scheme and improve the prediction performance

Future work:

- Collect more reliable negative samples
- Consider about bulges in the target sites
- Develop a complete sgRNA designing tool with the on-target cutting efficiency prediction with off-target site detection

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