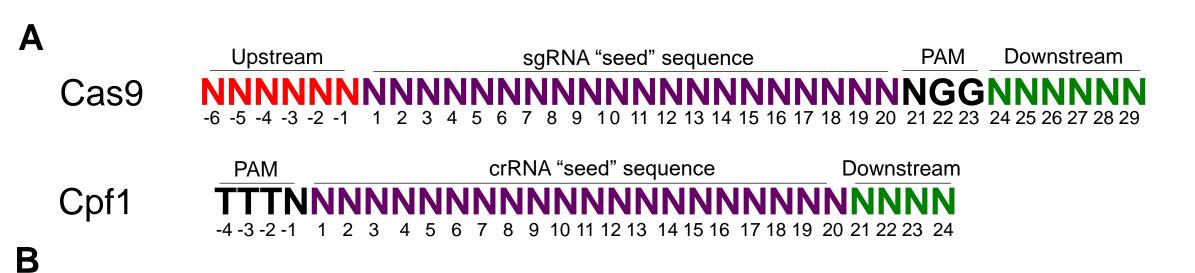
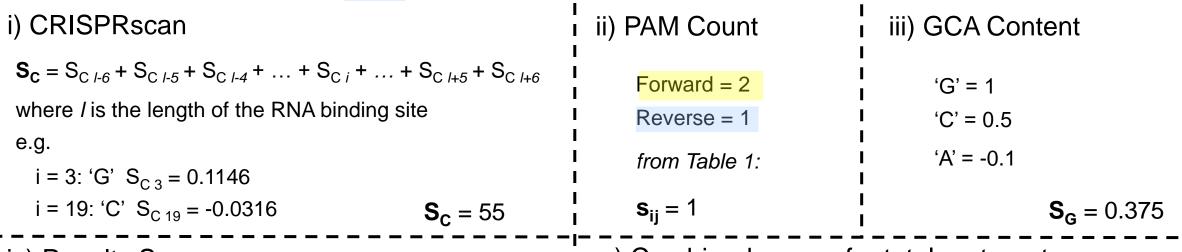
Supplementary Figure S1. (A) Nucleotide indexing of the target sequences for Cas9 and Cpf1 used in the development of the CASPER on- and off-target algorithms. 'N' stands for any nucleotide. **(B)** Demonstration of the on-target algorithm on a sample target sequence. The on-target score has a range of 0-100, with 100 being most active. **(C)** Demonstration of the off-target algorithm on a sample off-target sequence. The off-target has a range of 0-1, with 1 indicating a high probability of off-target activity.



Example target sequence:

TTTGGAGAGCCAAGGATTCGGGATTCTCGGCTTCCA



$$P = \begin{cases} S_{ij}S_G & S_{ij} > 1 \\ S_{ij} - S_G/5 & S_{ij} = 1 \end{cases}$$
 because $S_{ij} = 1$:
$$P = S_{ij} - S_G/5$$

$$P = 0.925$$

v) Combined scores for total on-target score

$$S_{C,P} = \frac{S_C}{P} = \frac{55}{0.925} = 59.46$$

Target sequence

Off-target sequence

GAGCCAAGGATTCGGATTCTCGG GAGCGGAGGATTCGGATACTGGG

20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2

i) Matrix	ii) Continuous	l iii) Step	iv) On-target score	v) Final off-target score
$S_{H3,TA} = 0.434$	$S_{T3} = 0.333$	$S_{S3} = 0.1$	ratio	
$S_{H15,AG} = 0.020$	$S_{T15} = 0.067$	$S_{S15} = 0.0125$	$S_{C,P RNA} = 59.5$	$\left(\sqrt{S_H} + S_T\right)R^2S_S^6$
$S_{H16,CG} = 0.113$	$S_{T16} = 0.063$	$S_{S16} = 0.0125$	$S_{C,PDNA} = 62.9$	$\mathbf{S}_{H,T,S} = \frac{\mathbf{A}_{H,T,S}}{4}$
Multiply all subscores (eqn. 6):	Sum all subscores for eqn. 7:	Sum all subscores for leqn. 8:	Divide RNA score by DNA score:	= 0.110
$S_{H} = 0.001$	S _S = 0.870	$S_T = 0.875$	R = 1.057	
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