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CRISPy-web: An online resource to design sgRNAs for CRISPR applications

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ABSTRACT

CRISPR/Cas9-based genome editing has been one of the major achievements of molecular biology, allowing the targeted engineering of a wide range of genomes. The system originally evolved in prokaryotes as an adaptive immune system against bacteriophage infections. It now sees widespread application in genome engineering workflows, especially using the *Streptococcus pyogenes* endonuclease Cas9. To utilize Cas9, so-called single guide RNAs (sgRNAs) need to be designed for each target gene. While there are many tools available to design sgRNAs for the popular model organisms, only few tools that allow designing sgRNAs for non-model organisms exist. Here, we present CRISPy-web (http://crispy.secondarymetabolites.org/), an easy to use web tool based on CRISPy to design sgRNAs for any user-provided microbial genome. CRISPy-web allows researchers to interactively select a region of their genome of interest to scan for possible sgRNAs. After checks for potential off-target matches, the resulting sgRNA sequences are displayed graphically and can be exported to text files. All steps and information are accessible from a web browser without the requirement to install and use command line scripts.

1. Introduction

"Clustered regularly interspaced short palindromic repeats" (CRISPR) and their associated RNA-guided endonucleases are bacterial adaptive immune systems protecting the bacteria from infections with bacteriophages.[1] The biotechnological application of this system is currently revolutionizing molecular biology and provides new opportunities for synthetic biology applications.[2] The CRISPR system allows targeted genome engineering of bacteria,[3–5] and also eukaryotes including yeast,[6,7] plants,[8] human cell lines[9] and many more. Although several alternative CRISPR systems have recently been described (e.g., References 10–13), most CRISPR systems for genome engineering are based on the *Streptococcus pyogenes* type II CRISPR/Cas9 system. Cas9 is an RNA-guided endonuclease, which introduces double strand DNA breaks at positions that are complementary to a crRNA sequence that binds to Cas9 in a duplex with a tracrRNA. It has also been demonstrated that Cas9 accepts artificially fused crRNA:tracrRNA-hybrids termed "single guide RNAs" (sgRNAs) to direct it to the target cut sites.[14] Thus CRISPR can be used as an "*in vivo* programmable restriction enzyme," which cuts the target DNA within an exactly defined target sequence determined by the sgRNA.

One essential step in the design of CRISPR experiments is to identify suitable sgRNA sequences within the target gene(s), which have to fulfill certain conditions: (i) the 20 bp target sequence has to be directly upstream of a "protospacer adjacent motif" (PAM). For the *S. pyogenes* PAM, this motif is "NGG", (ii) it is desirable that the sequence of the target motif is unique within the genome of the organisms to prevent off-target activity, *i.e.* the cleavage of the chromosome at wrong positions.

Many programs and web-servers exist to assist biologists in finding such CRISPR/Cas9 target sites. However, most of these tools (e.g. CCTop,[15] CHOPCHOP,[16] CRISPR Design,[17] WU-CRISPR,[18] WGE CRISPR Finder,[19] and CRISPy CHO[20]) are limited to a narrow set of – mostly eukaryotic – target genomes of model organisms. Only very few tools (e.g. sgRNAcas9[21]) allow running Cas9 target searches on

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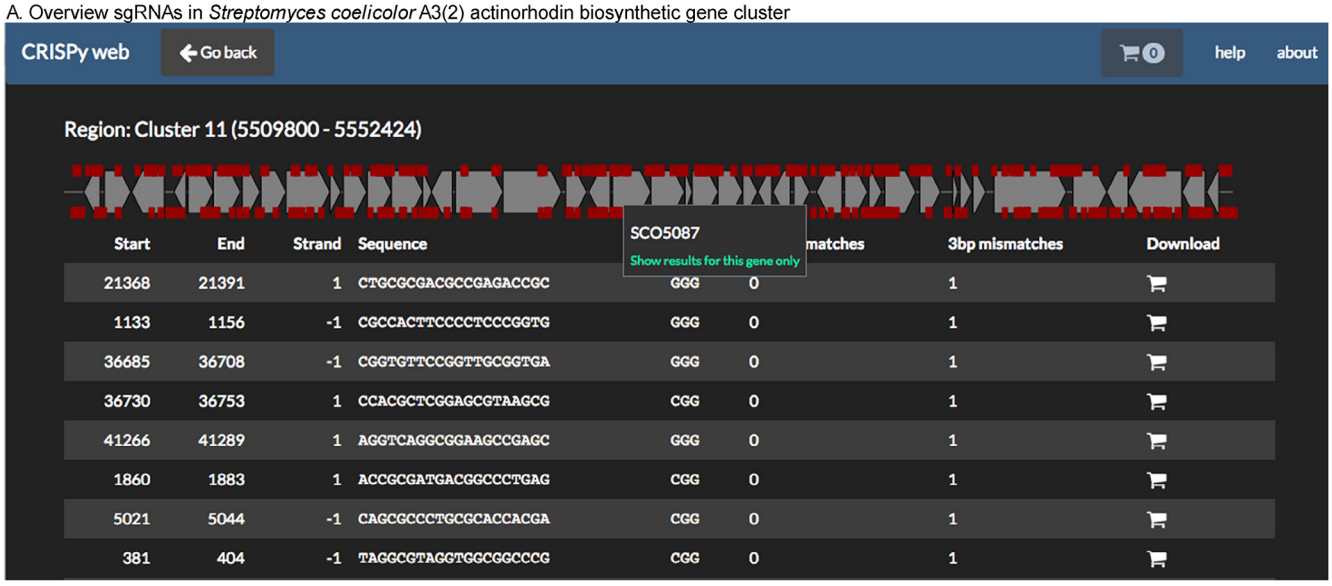
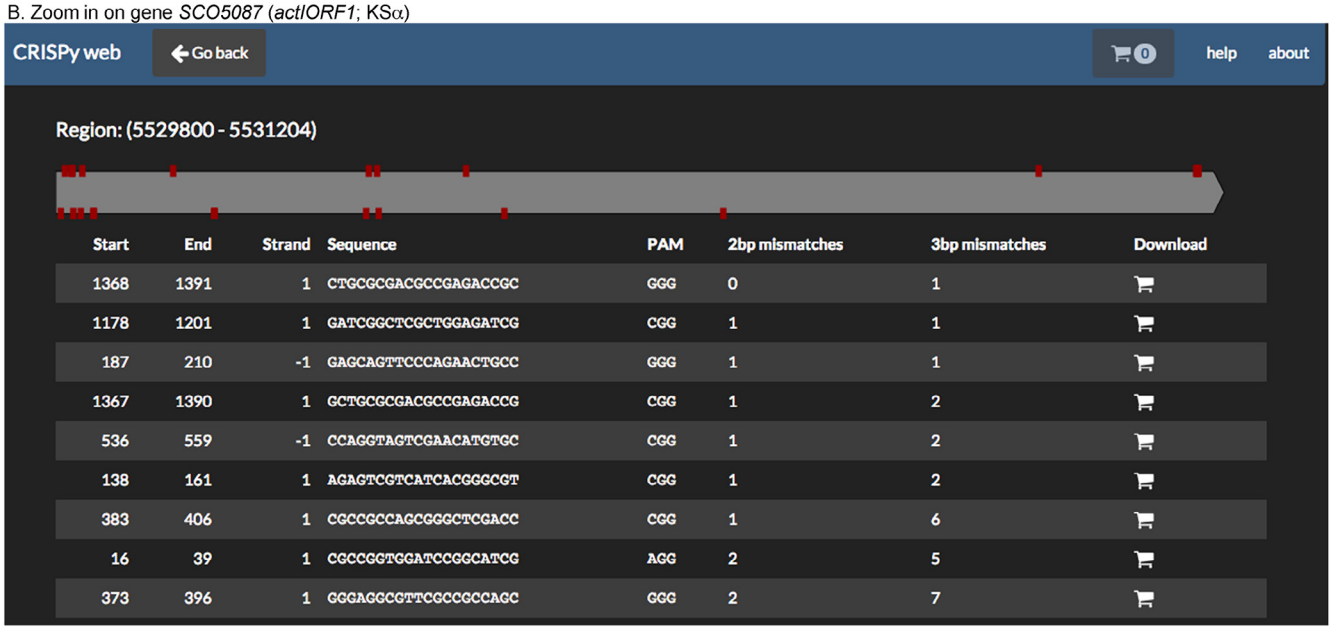
A. Overview sgRNAs in *Streptomyces coelicolor* A3(2) actinorhodin biosynthetic gene cluster



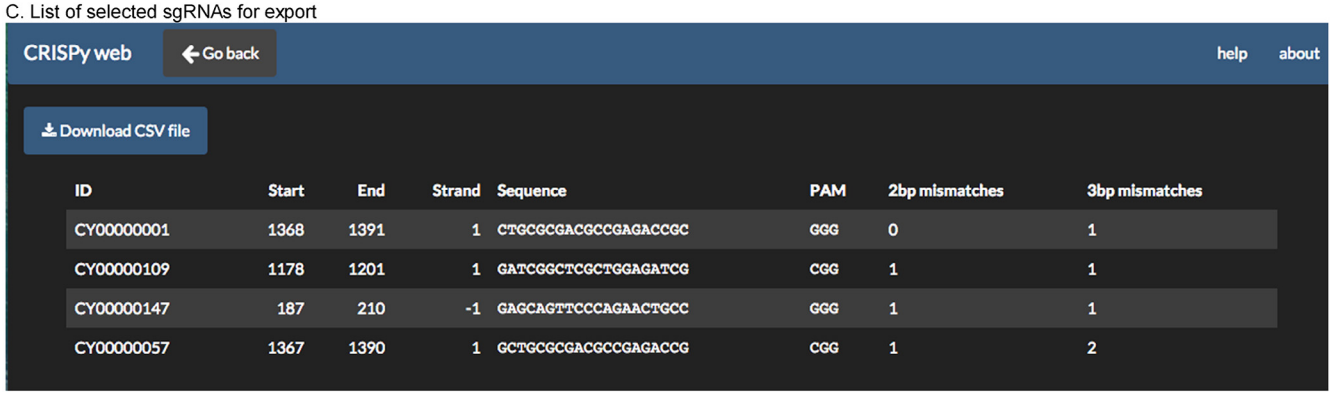
Start	End	Strand	Sequence		matches	3bp mismatches	Download
21368	21391	1	CTGCGCGACGCCGAGACCGC	GGG	0	1	
1133	1156	-1	CGCCACTTCCCCTCCCGGTG	GGG	0	1	
36685	36708	-1	CGGTGTTCCGGTTGCGGTGA	GGG	0	1	
36730	36753	1	CCACGCTCGGAGCGTAAGCG	CGG	0	1	
41266	41289	1	AGGTCAGGCGGAAGCCGAGC	GGG	0	1	
1860	1883	1	ACCGCGATGACGGCCCTGAG	CGG	0	1	
5021	5044	-1	CAGCGCCCTGCGCACCACGA	CGG	0	1	
381	404	-1	TAGGCGTAGGTGGCGGCCCG	CGG	0	1	

B. Zoom in on gene *SCO5087* (*actIORF1*; KSα)



Start	End	Strand	Sequence	PAM	2bp mismatches	3bp mismatches	Download
1368	1391	1	CTGCGCGACGCCGAGACCGC	GGG	0	1	
1178	1201	1	GATCGGCTCGCTGGAGATCG	CGG	1	1	
187	210	-1	GAGCAGTTCCCAGAACTGCC	GGG	1	1	
1367	1390	1	GCTGCGCGACGCCGAGACCG	CGG	1	2	
536	559	-1	CCAGGTAGTCGAACATGTGC	CGG	1	2	
138	161	1	AGAGTCGTCATCACGGGCGT	CGG	1	2	
383	406	1	CGCCGCCAGCGGGCTCGACC	CGG	1	6	
16	39	1	CGCCGGTGGATCCGGCATCG	AGG	2	5	
373	396	1	GGGAGGCGTTCGCCGCCAGC	GGG	2	7	

C. List of selected sgRNAs for export



ID	Start	End	Strand	Sequence	PAM	2bp mismatches	3bp mismatches
CY00000001	1368	1391	1	CTGCGCGACGCCGAGACCGC	GGG	0	1
CY00000109	1178	1201	1	GATCGGCTCGCTGGAGATCG	CGG	1	1
CY00000147	187	210	-1	GAGCAGTTCCCAGAACTGCC	GGG	1	1
CY00000057	1367	1390	1	GCTGCGCGACGCCGAGACCG	CGG	1	2

Fig. 1. Example output of a CRISPy-web run to identify sgRNAs in the actinorhodin biosynthetic gene cluster of *Streptomyces coelicolor* A3(2) (NCBI GenBank ID: NC_003888.3). (A) Overview of sgRNAs identified in the complete actinorhodin gene cluster. (B) Zoom view of *SCO5087* (*actIORF1*). (C) List of sgRNAs selected for export.