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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. The biotechnological application of this system is currently revolutionizing molecular biology and provides new opportunities for synthetic biology applications. The CRISPR system allows targeted genome engineering of bacteria, and also eukaryotes including yeast, plants, human cell lines 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. 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 “protr spacer 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, CHOPCHOP, CRISPR Design, WU-CRISPR, WGE CRISPR Finder, and CRISPy CHO) are limited to a narrow set of – mostly eukaryotic – target genomes of model organisms. Only very few tools (e.g. sgRNAcas9) allow running Cas9 target searches on
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, KS). (C) List of selected sgRNAs for export.
user‑specified genomes. Unfortunately, to the best of our knowledge, none of these generally applicable tools are provided as user‑friendly web services, which can be used by non‑computer scientists.

Here, we therefore present a web server implementation of the sgRNA prediction software CRISPy (http://crispy.secondarymetabolites.org/) that was originally designed for CRISPR‑based engineering of Chinese hamster ovary (CHO) cells. It is implemented as a standalone web application for Cas9 target prediction that can design sgRNA for any gene/regional interest in user‑submitted genome data. In addition, CRISPy‑web tightly connects with the popular natural product prediction tool antiSMASH22–24 to assist researchers working on microbial secondary metabolites in leveraging the CRISPR/Cas9 system. Using antiSMASH or working on natural products of course is not a prerequisite for using CRISPy‑web.

2. Design and implementation

CRISPy‑web consists of multiple decoupled service components tied together by a message queue. The main services are the CRISPy worker service, the web application programming interface (API) server and the web user interface (UI). Sequences to be scanned for Cas9 target sites can be uploaded directly (in GenBank format) or transferred from antiSMASH22–24 by specifying the antiSMASH job ID. In the next version of antiSMASH, a direct link for sgRNA prediction software CRISPy (http://crispy.secondarymetabolites.org/) will also be implemented.

2.1. CRISPy worker service

Two steps are performed by the worker service: preparing a sequence for analysis and then scanning and ranking Cas9 target sites. In the preparation step, the input files are parsed and – if the files were transferred from antiSMASH – annotated with secondary metabolite gene cluster locations using Biopython.25

Based on the CRISPy algorithm initially used in Ronda et al.,20 the scanning step searches for the ‘NGG’ PAM sequence on both the forward and the reverse strands of the selected region of interest, counting the number of direct off‑target hits for sequences with the same 13 bp core sequence upstream of the PAM. It then calculates the number of off‑target hits containing 0, 1, 2, or 3 bp mismatches on the whole genome for the 13 bp sequence by considering all possible combinations.

After collecting all possible Cas9 targets, sites that have direct off‑target matches, sites with off‑target matches with a single base pair mutation, or sites with more than ten off‑target matches with a two base‑pair mutation are discarded. All these hits are stored in a GenBank file. To further reduce the number of sites displayed on the user interface, the targets are now sorted by 2bp mismatch of the upper right will track the number of selected sgRNAs.

A click on the download basket icon will display the download overview page (Fig. 1C). There, the selected sgRNAs can be verified again. A click on the “Download CSV file” button will start the download of all selected sgRNAs in comma-separated value format. Files in this format can easily be used in a spreadsheet application, processed for downstream computational analysis or oligonucleotide orders.

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References


