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RESEARCH ARTICLE

CASPER: An Integrated Software Platform for Rapid Development of CRISPR Tools

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Abstract

Both academic and enterprise software solutions exist for designing CRISPR targets. They offer advantages when designing guide RNAs (gRNAs) but often focus on a select number of model organisms. Those that offer a wide variety of organisms can be limited in support of alternative endonucleases and downstream analyses such as multitargeting and population analyses to interrogate a microbiome. To accommodate broad CRISPR utilization, we developed a flexible platform software *C*RISPR *A*ssociated *S*oftware for *P*athway *E*ngineering and *R*esearch (CASPER) for gRNA generation and analysis in any organism and with any CRISPR-Cas system. CASPER combines traditional gRNA design tools with unique functions such as multiple Cas-type gRNA generation and evaluation of spacer redundancy in a single species or microbiome. The analyses have implications for strain-, species-, or genus-specific CRISPR diagnostic probe design and microbiome manipulation. The novel features of CASPER are packaged in a user-friendly interface to create a computational environment for researchers to streamline the utility of CRISPR-Cas systems.

Background

The application of CRISPR-Cas systems has revolutionized biological research and industry alike.[1–9] The facile genome editing capabilities of CRISPR-Cas lie in its ability to specifically home in on a target sequence using a 20–40 bp RNA sequence matched to a site on the genome/transcriptome. Most systems are further controlled with a protospacer adjacent motif (PAM) on either the 5′ or 3′ end of the sequence that interacts directly with the Cas protein complex and serves as a first checkpoint for substrate binding.[4,10–12] The immense design space of targeting virtually any location on any genome with CRISPR-Cas machinery necessitates the development of rational sequence design tools to expedite experimental design.[13,14]

Central to the creation of guide RNA (gRNA) sequences is the ability to score on- and off-target activities. Sequence similarities of gRNAs presumably determine whether an RNA-guided endonuclease can successfully identify a target site on a genome and, conversely, whether it will bind at off-target locations with high sequence similarity.[15] A variety of algorithms have worked off this premise[16–18]; however, further investigation into the fundamental biochemistry of spCas9's mechanism of action has illuminated other important features for analyzing on- and off-target binding and cleavage events.[8,18,19] For instance, PAM density and the formation of guanine quadruplex motifs have been shown to affect spCas9 activity.[20]

In addition, biomechanistic studies have provided insight into the kinetic and thermodynamic parameters associated with CRISPR-Cas enzymatic activity.[21–23] Finally, the increasing number of large assays to determine rules of activity is foundational to both identifying and confirming relevant features contributing to CRISPR-Cas activity.[15,17,18,24–29] Therefore, incorporating these features in the gRNA design algorithms helps select efficient guides for precise genome editing.

Currently, many such tools exist that employ either their own developed scoring algorithms or employ one or a series of previously developed algorithms to score identified guide sequences. Although this scoring is a core element of any design tool, there exists a growing need for platforms with increased functionality as the applications of CRISPR-Cas diversify. The first wave of increased functionalities came with the advent of

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engineered Cas nucleases with unique PAMs and specificities, creating new applications such as CRISPR interference/activation accompanied by experimentally validated rules for designing guides.[30–33]

The rapid adoption of modified Cas proteins for base editing and the associated rules for designing appropriate guide sequences further prompted the incorporation of new algorithms in existing software design platforms.[25,34–36] Finally, use of CRISPR gene editing has rapidly expanded to interrogate microbiomes beyond single species.

This article introduces *C*RISPR *A*ssociated *S*oftware for *P*athway *E*ngineering and *R*esearch (CASPER), an integrated software platform that contains features for designing CRISPR-Cas guides for diverse applications in nonmodel organisms and microbiomes. CASPER employs its own algorithms for on- and off-target analyses and has the unique capability of employing user-specified mismatch weighting parameters.

In addition, CASPER provides end users desired simplicity and flexibility in performing multitargeting analyses to exploit spacer redundancy in addition to conventional multiplexing analyses and designing gRNAs for editing either single or a consortium of organisms beyond select model species. Integration of the NCBI database in CASPER facilitates CRISPR-mediated manipulations for 66,000+ genomes with multiple Cas endonucleases and user-defined PAM specification options.

Results

CASPER infrastructure

To streamline analyses of CRISPR utility, we created a graphical user interface (GUI) software, CASPER, to interrogate a single or consortia of species (Fig. 1A). CASPER infrastructure is built using a modular framework. The current version comprises four modules, including New Genome, Target Finder, Multitargeting, and Population Analysis, which possess novel functionalities and can be expanded for future development (Fig. 1B). Any analysis must start with New Genome to create a database of gRNA sequences stored in a *.cspr file for unique sequences and a *.db file for degenerate sequences (Fig. 1B).

In the Main Program, these files are used to perform the on-target and off-target analyses of gRNAs in the Target Finder module, identify the spacer redundancy in the Multitargeting module, and enable gRNA design to target-specific species in a microbiome in the Population Analysis module (Fig. 1B). With the inputs of organism(s), a type of endonuclease, and gene target(s) of interest, CASPER outputs the most likely active gRNA targets at the selected loci, with associated on- and off-target scores (Supplementary Fig. S1).

Organism and endonuclease support in the New Genome module

CASPER is designed to support genome editing across any organism through the New Genome module (Fig. 2A). Using the NCBI tool within this module (Fig. 2B), users may download and use any genomic sequence files (FASTA/FNA extension) and annotation files (GBFF extension) available in the GenBank and RefSeq collections of the vast NCBI database. Alternatively, users may upload a custom genome sequence themselves. Because of the genomic sequence flexibility, CASPER allows researchers to investigate the use of CRISPR-Cas systems to genetically modify communities of species and metagenomic assemblies.

In addition to multiple genome support, CASPER can generate guides for natural and engineered Cas9 orthologs[37] and other Cas endonucleases such as AsCas12[14] in the built-in endonuclease database within the New Genome module, and even novel user-defined endonucleases using the ''Define New Endonuclease'' toolbox available in the Main Program. The latter functionality allows CASPER to be used for designing guide sequences that harness an organism's native CRISPR machinery. With the selection of annotated genome and endonuclease, running the New Genome module of CASPER will create databases of gRNA sequences for downstream analysis stored in the *.cspr and *.db files using the SeqFinder algorithm (Supplementary Table S1).

gRNA selection and library generation in the Target Finder module

The Target Finder module in the Main Program (Fig. 3A) is designed to find gRNAs for a target gene(s) and assign them with relative on-target and off-target scores using our previously developed CASPERon and CASPER-off algorithms, respectively.[38] Within the Target Finder module, using either keyword or position-based searches, users can precisely find the desired region(s) of the genome they wish to target (Fig. 3A). Once a gene or locus has been selected, users may continue with either bespoke or batch gRNA generation. This modular design enables users to perform multiplexing analyses to find multiple gRNAs targeting multiple loci. Seamless integration with annotation files makes CASPER's gene targeting fast and facile.

In addition to on- and off-targeting analyses, the View Targets GUI of CASPER provides several features to aid in custom gRNA selection, including gRNA visualization within the gene of interest, on-target and off-target scores, 5′ motif filtering, and cotargeting analysis (Fig. 3B). The cotargeting feature identifies sequences that have compatible PAM sequences for multiple

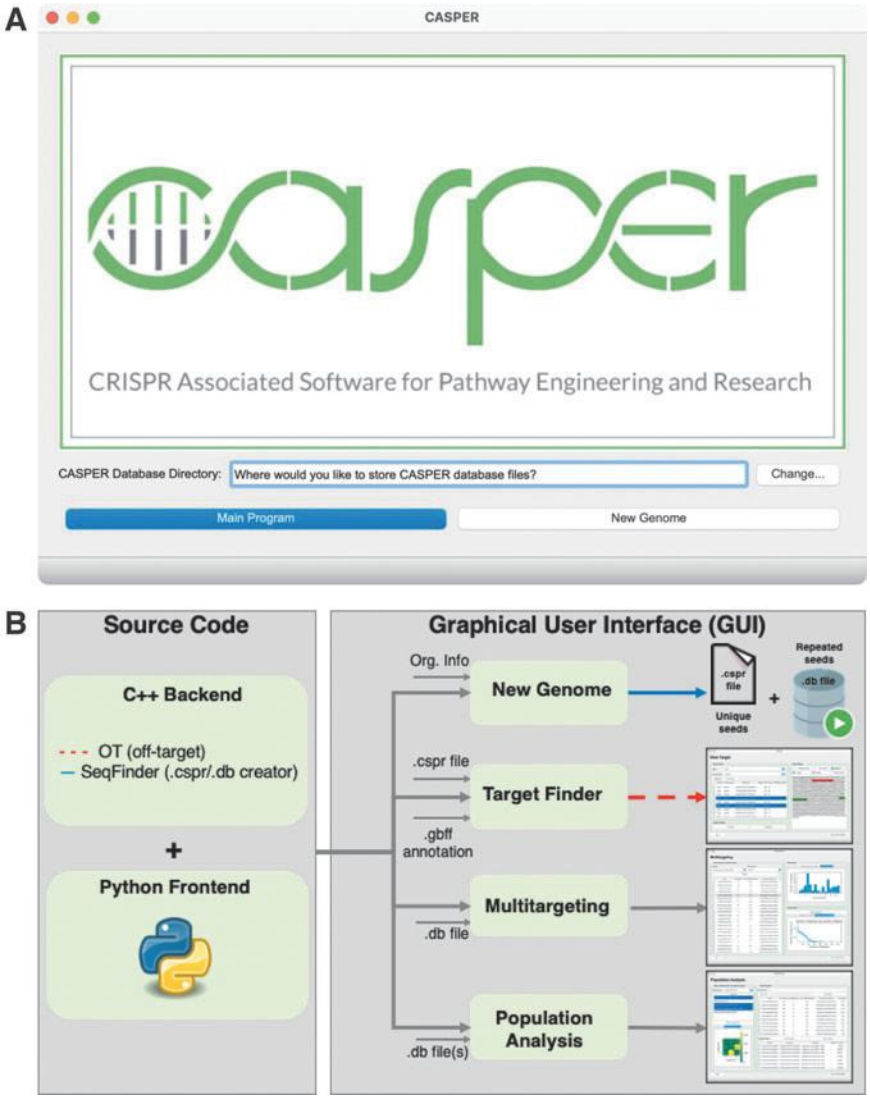

FIG. 1. **(A)** GUI of CASPER. **(B)** CASPER infrastructure. CASPER is written in C++ in the backend computation to process genome sequences and perform on- and off-targeting analyses. For the frontend computation and GUI, Python is used. CASPER contains four modules, including New Genome, Target Finder, Multitargeting, and Population Analysis. CASPER, *C*RISPR *A*ssociated *S*oftware for *P*athway *E*ngineering and *R*esearch; GUI, graphical user interface.

endonucleases, thereby minimizing variables when performing experiments with multiple Cas systems to identify promising enzymes in the application of choice.

When working with Cas13 endonucleases or other Cas systems that have a permissive protospacer flanking sequence, users are encouraged to use CASPER with a more restrictive PAM requirement such that guides can be designed as inherently cotargeting. This not only saves memory by decreasing database file size but also allows near-identical guide sequences to be used with additional Cas systems. CASPER makes this process trivial with facile selection of multiple guides for export to a comma-separated value (CSV) file.

Owing to the usefulness and increasing practice of CRISPR screening techniques, as well as the hit-or-miss nature of gRNAs, it is extremely beneficial to facilitate the generation of vast quantities of high-performing guides with minimal time expenditure. CASPER's Generate Library feature robustly addresses this issue by providing the end-user means to quickly create and export customizable libraries of gRNAs (Figs. 3A, 3C).

Users are given the option to select up to 10 gRNAs per locus, the region within the loci from which to pull them, minimum on-target and maximum off-target scores, and several other options. The library is then exported to a CSV file, making alteration or ordering of guides as

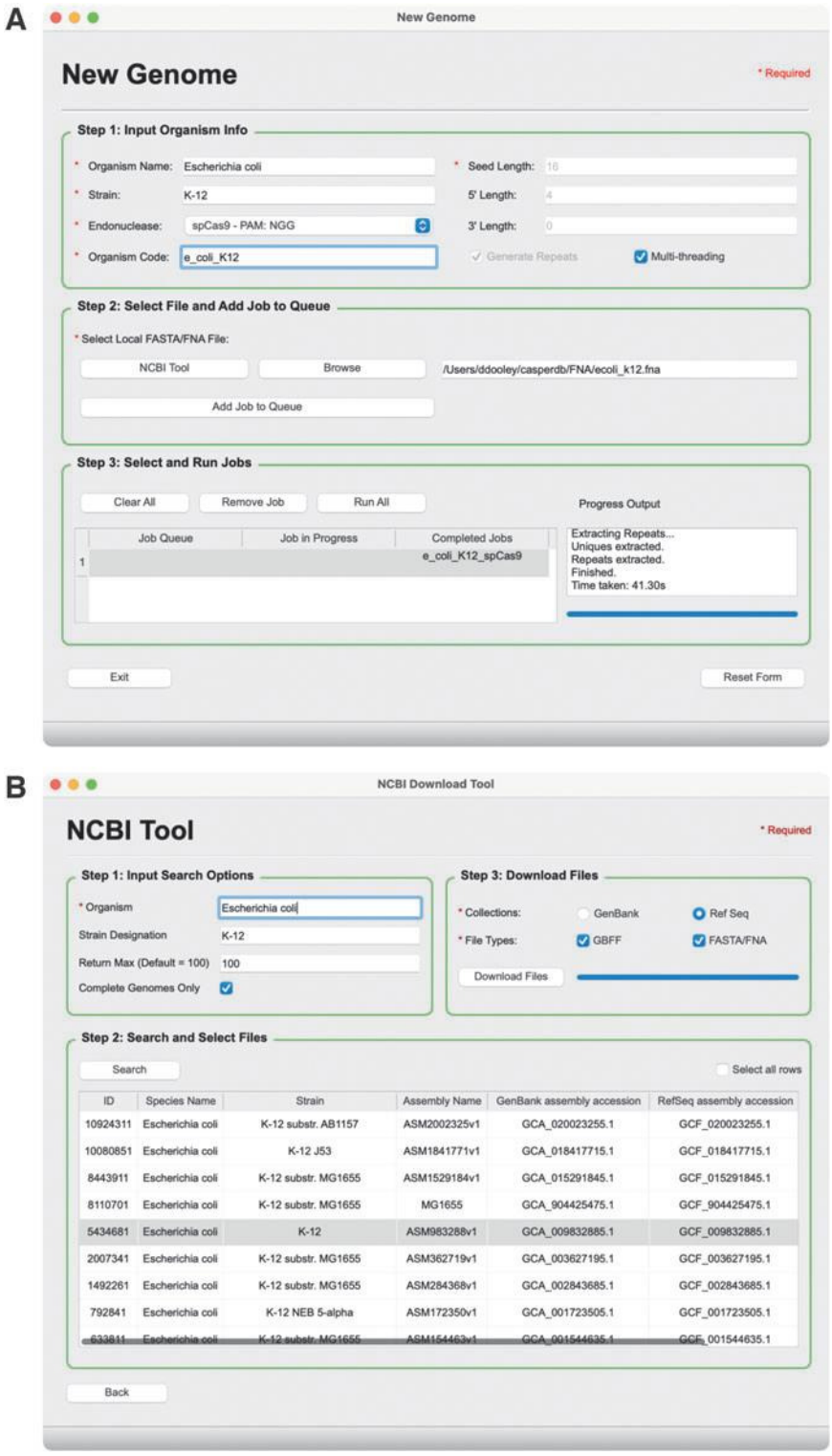

FIG. 2. New Genome module. **(A)** The New Genome GUI allows users to create a database of gRNA sequences (unique and repeat gRNA databases) for a specified genome and endonuclease. **(B)** The NCBI Tool GUI enables users to download a genome(s) from NCBI. gRNA, guide RNA.

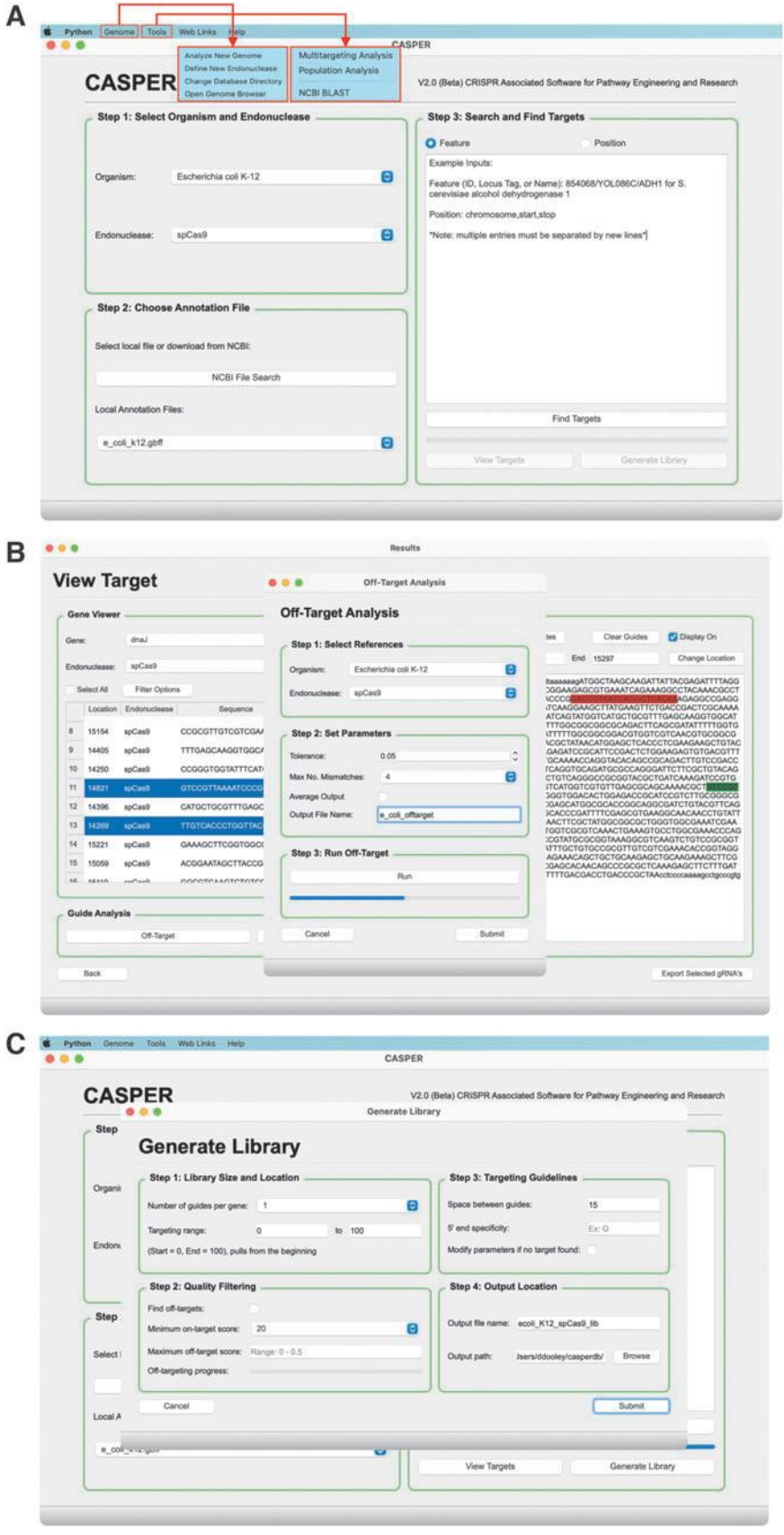

FIG. 3. Target Finder module. **(A)** The Target Finder GUI enables users to identify gRNA libraries to target a specific DNA sequence(s) or genes. Multiple loci can be selected for multiplexing analysis. **(B)** The View Targets GUI analyzes on- and off-target activities of gRNA libraries and export the results. Cotargeting feature can also be performed to identify a guide that can be compatible with different endonucleases. **(C)** The Generate Library GUI selects a library of gRNAs that target a DNA sequence or gene filtered by their locations, on- and off-target activities.

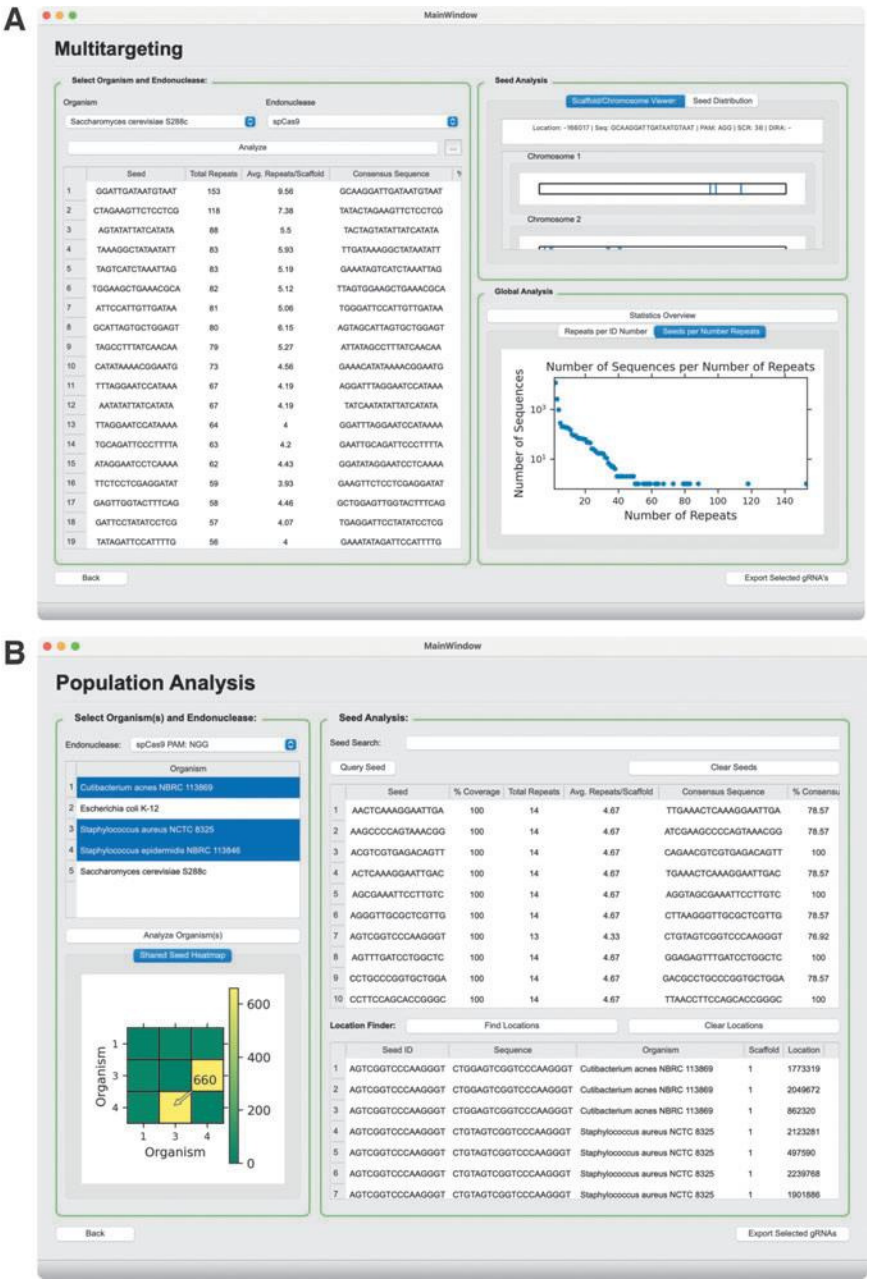

FIG. 4. **(A)** The Multitargeting module identifies a library of single guides targeting multiloci. **(B)** The Population Analysis module identifies a library of guides that target one or multiple loci within one or more species within a microbiome.

effortless as possible. We envision this feature as a useful resource for the scientific community that will help bolster efficiency and productivity of various CRISPR assays.

Multitargeting and Population Analysis modules

The manipulation of multiple genes and entire operons is the primary focus of many metabolic engineering and synthetic biology endeavors, and CRISPR tools offer powerful resources to enable such activities.[1] As such, CASPER's user interface focuses on streamlining the design process for multigene disruption and/or insertion. Although the ability to design gRNAs to target genes in model organisms is incredibly useful but hardly novel,[2] CASPER distinguishes itself through two features that are particularly useful when dealing with nonmodel organisms: multitargeting and population analyses.

CASPER's Multitargeting module is a robust platform for targeting multiple sites across the genome with identical or similar gRNA sequences using the Multitargeting algorithm (Supplementary Table S2). Although multiple gRNAs are used to target multiple loci in multiplexing, multitargeting utilizes one guide to target multiple loci. This analysis can be used to interrogate common motifs across a genome for manipulation or for integration of multiple copies of genes using a single gRNA template (Fig. 4A). It can also identify conserved motifs in genome(s) representing potential sites for global cellular process manipulation (e.g., stress response, transposons).[38]

The rising interest in manipulation of microbiomes prompted the population analysis feature of CASPER (Fig. 4B). In the Population Analysis module, users begin by selecting two or more organisms and clicking ''Analyze Population.'' The Population Analysis algorithm (Supplementary Table S3) calculates conserved seeds at both pairwise and population levels. Shared motifs may be viewed in greater detail to elucidate their exact locations within each organism and which guides are best suited for targeting them. An overview of the targeting relationships of all species within the community is represented by a pairwise heatmap of shared genomic targets.

By coupling population analysis with single gRNA analysis on the same *.cspr database files, users may find either degenerate gRNAs capable of editing an entire community at once or specific gRNAs that selectively edit a single species within a complex consortium. This approach has immediate application for CRISPR antimicrobial gRNA design,[39–41] wherein broad-spectrum gRNAs may be designed to target all strains of a pathogen of interest (POI), or strain-specific gRNAs could be found to eliminate a POI amid a highly similar commensal population. The gRNA design for cell populations opens yet another dimension in the scope of genome editing with CRISPR-Cas for a rapidly evolving field of study.

Discussion

Various computational tools have been developed for designing CRISPR targets (Supplementary Table S4). They each offer advantages when designing gRNAs but very often focus on a select number of model organisms. Those that do offer a wide variety of organisms can be limited in their support of alternative endonucleases and further downstream analyses such as multitargeting and population analyses.

In addition to being the only all-in-one GUI-based CRISPR design software for single and batch gRNA generation with on- and off-target scoring for any organism–endonuclease pair, CASPER possesses several novel features that distinguish it from other solutions, namely the abilities to cotarget genomic loci with compatible endonucleases and simultaneously target multiple sites in single or consortia of cell populations, a useful resource for metabolic engineering and synthetic biology applications.

The version of the software presented here is designed with inherent flexibility to accommodate both the input of users and expanding research into more refined studies of CRISPR-Cas activity across the organism/endonuclease design space. A defining feature of CASPER is its population analysis capability. We envision that as greater understanding of microbiome dynamics becomes available, this tool can evolve to serve the research community's needs, particularly in pairing appropriate design with experimental tools. In addition, a priority is put on maintaining compatibility with expanding databases, particularly for metagenomes, as this is critical for maintaining the platform as a cutting-edge tool.

In summary, CASPER exists at the intersection of powerful back-end algorithms and simple, intuitive GUIs, making it a potent CRISPR design tool for specialist and nonspecialist alike. The addition of multitargeting and population analyses further differentiates CASPER from its contemporaries, opening the door to new and exciting analyses for multiple strains of a single viral or bacterial species, as well as bacterial communities and metagenomes. Immediately, these analyses have implications for strain-, species-, or even genus-specific CRISPR diagnostic probe design and microbiome manipulation. We expect CASPER to empower these and other applications of CRISPR biology, expanding their impact and utility.

Materials and Methods

Algorithms

The current CASPER program contains four core algorithms: SeqFinder, CASPERon/off, Multitargeting, and Population Analysis. The SeqFinder algorithm used in the New Genome module processes a genomic sequence file to extract, sort, store, and retrieve gRNA sequences through indexing and string compression. The analysis of CASPER for a new genome runs at $O(N^2)$ speed, with N being the genome size.

This algorithm has been optimized from our previous version[38] and adapted for the CASPER GUI to process large genomes' data and streamline users' input and output data. For instance, the employment of divide-and-conquer strategy in the SeqFinder algorithm to index large genomes resulted in a reduction of peak memory usage from 16+ GB down to 2 GB for human genomes, and a decrease in processing time from 360 to 30 min. The details of pseudocode for the SeqFinder algorithm are shown in Supplementary Table S1.[38]

The CASPERon and CASPERoff algorithms are core to the Target Finder module. These algorithms are derived from our previous versions[38] and adapted to fit the CASPER GUI to streamline users' input and output data. In brief, an evolutionary optimization is employed to enable multivariate regression on a large experimental data set, taking into account (1) the CRISPRscan features experimentally identified to be present in highly active guide sequences,[26] (2) the density of the PAM in question across the guide sequence,[20] (3) the propensity for guanines (and to a lesser extent cytosines) over adenines in the gRNA sequence that has been shown to be a factor in gRNA stability,[17,42] and (4) nucleotide mismatches.[15,43] A workflow of CASPER's target identification implementation is detailed in Supplementary Fig. S1.

Both the Multitargeting and Population Analysis algorithms use the output of the SeqFinder algorithm to process the unique and repeat sequences. These algorithms have been modified to fit the CASPER GUI, which manages the data inputs and outputs and downstream analyses. Details of the Multitargeting and Population Analysis algorithms are shown in Supplementary Tables S2 and S3, respectively.

Computation and programming languages

CASPER is designed as a standalone program capable of operating on Linux, Windows, and Mac OS X operating systems. CASPER provides an easy-to-use GUI for a suite of novel algorithms we previously developed for prediction of on- and off-target activities for gRNA design, as well as performing multitargeting and population analyses.[38] Algorithms for the creation of databases and identification of on- and off-targets have been implemented in C++. C++ was chosen due to the substantial memory management necessary for the creation of organism databases. The GUI is entirely implemented using the PyQt5 Python package, figures are generated with the popular Python graphing package, matplotlib, and front-end data handling/parsing is handled natively within Python.

CASPER availability

CASPER can be downloaded as a standalone application through (https://github.com/TrinhLab/CASPERapp). Documentation for installing CASPER and using the software is provided in Supplementary Material S1. In addition, video tutorials are available at the Trinh Lab YouTube channel (Supplementary Material S1). The YouTube link is: (https://www.youtube.com/channel/UC3qfnXxv4KqKlo1j_BSx-MQ).

Authors' Contributions

C.T.T. conceived and supervised the project. B.M. conceptually developed the back-end algorithms used in CASPER. T.F. did most of the coding (front-end and back-end) and assisted in the design of CASPER's GUIs. D.D. designed CASPER's overall workflow and GUI, managed the production of the software, and helped with coding. J.H. helped with coding the annotation file parsing/search and Generate Library features. B.M., D.D., and C.T.T. wrote the article.

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Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Material S1
Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Table S4
Supplementary Figure S1

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