Mini Review
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Computational tools and resources for CRISPR/Cas 9 genome editing method

Abstract

CRISPR/Cas9, the popular genome editing method, has wide applications ranging from genetic engineering of plants, modifying bacterial genomes for production of therapeutic, agricultural, industrial bioproducts. It would be an understatement to claim that it has indeed revolutionized the field of gene and genome editing. To aid the researchers in designing the optimal sequences for CRISPR/Cas9 experiments, a multitude of bioinformatics tools and resources have been developed and are currently used. This review aims to provide an up to date list of tools, web servers, databases, and other resources that can be used for editing the genome. We hope that such a compilation will enable researchers to select the resources based on functionality, ease of use, availability, and specific research needs.

Keywords: CRISPR/Cas9, genome editing, bioinformatics, resources, database, computational tools

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Abbreviations: CRISPR-Cas9, clustered regularly inter-spaced palindromic repeats-CRISPR associated proteins 9; crRNA, CRISPR RNA; DSB, DNA double helix break; sgRNA, single guide RNA; TALENs, transcription activator-like effectors nucleases; tracrRNA, transactivating CRISPR RNA; ZFNs, zinc finger nucleases

Introduction

From an evolutionary point of view, archaea and bacteria have evolved to survive and blossom as communities in active environments that are, in general, stressful and unpredictable. Specifically, the abundance of viruses attacking microorganisms is a constant threat; in addition, the mutation and recombination rates in viruses make them fast-evolving predators. To sustain against such predators, archaea and bacteria have evolved to have a new multilayered defense system that has been identified as CRISPR/Cas9.[1] Consequently, many labs have tapped into the potential uses for this method and active research to unravel the mechanism has paved a unique path in the development of targeted genome editing techniques.[2] Among the various genome editing methods, TALENs, ZFNs, and CRISPR-Cas9 are the three foremost methods.[3]

The advantage of CRISPR as a more reliable method is the ease in the creation of a break in the helical strand using RNA-guided nucleases that involve canonical base pairing between the target DNA region and the RNA designed for binding to it. These inducible alterations in the genome provide a potential avenue for not only transferring traits in livestock and crops, but also to rectify diseased genes for human therapeutics with application in gene therapy. Another advantage is the non-dependency on protein-based system such as ZFNs and TALENs.[4]

CRISPR-Cas9 system is made of three components, viz: a small non-coding RNA called tracrRNA, a 20 nucleotide containing RNA sequence called crRNA that binds to the target DNA, and a RNA sequence repeat of 19-22 bases; and a Cas protein encoding operon. For simplification of the system for targeted mutations, a sgRNA with unique restriction sites for specific insertion of the target by combining endogenous tracrRNA and crRNA.[5]

CRISPR-Cas9 gene editing technology can generate transformative medicines in health sector such as the production of antimicrobials, animal health and therapeutic bio products. In the case of agricultural biotechnology, the CRISPR-Cas9 technology aids in unraveling of both functional and phenotypical data about target plants and enhancing their resilience against drought and disease. Also, through breeding crops with higher yields.[6] CRISPR-Cas9 technology is a valuable tool in biological research specifically to modify genes through knockout studies, inserting specific sequences, up-regulation and down-regulation of genes. The profound use of CRISPR-Cas9 technology has acknowledged by several researchers in generating accurate and efficient models of disease in cell lines, primary cells, and animal models.[7] CRISPR-Cas9 technology provides a precise method to improve industrially important microbial strains as cell factories (Eg: fermentation) for production of food, pharmaceutical, and biofuel products.[8]

CRISPR's extensive applicability via gene editing and its easy rules for designing sgRNA has provided an excellent opportunity for bioinformatics researchers to get an insight of various aspects of CRISPR mechanism using computational approach and development of software that identifies potential sgRNA sequences in genomes. Although, several design tools, web servers, and databases exist not all of them are similar. Specifically, each has an advantage over the other. Hence, to make the job easier for researchers around the globe, there is a need to keep an eye for various CRISPR design software based on some of the potential advantages, such as functionality, ease to use, its availability and more specifically research needs.

Software and web servers for designing sgRNA sequences

Table 1 lists the currently available software (standalone and web server) that used for designing sgRNA for CRISPR/Cas9 experiments. While previous reports have listed the available software/web server for CRISPR/Cas9 experiments, some of the resources are inaccessible, for unknown reasons. For example, the web servers ZiFiT, Cas9-Design, Jack Lin's CRISPR/Cas9 gRNA finder, and GT-Scan are no longer accessible for users. Among the 54 web servers listed in Table

1, more than 20 resources are available for public use since 2015, in comparison 13webservers have been reported in 2016, and most recently one web server (CRISPR-RT) has been published in 2017.

Additionally, there are 5 software that can use in the standalone mode, by either running a Perl script (sgRNAcas9) or via using an R code (caRpools) (Table 2). There also desktop based applications (DESKGEN) that can be utilized by molecular biologists for designing sgRNA sequences. The potential drawback to the standalone versions could be software compatibility, ease of usage, and additionally, some may be available for a fee.

Table 1 List of software and web servers for designing sgRNA sequences.

S. no	Tool name	Standalone/ Web server	Link	Reference
1	DESKGEN	Standalone	https://www.deskgen.com/landing/	9
2	sgRNAcas9	Standalone/command-line	http://www.biootools.com/	10
3	caRpools	Standalone/ command-line	http://github.com/boutroslab/caRpools	11
4	CRISPR Recognition Tool	Standalone	http://www.room220.com/crt/	12
5	CRISPRseek	Standalone	http://bioconductor.org/packages/release/bioc/html/CRISPRseek.html	13
6	CRISPRdirect	Web/online/graphic interface	http://crispr.dbcls.jp/	14
7	CRISPR RNA Configurator	Web/online/graphic interface	http://dharmacon.gelifesciences.com/ ch/gene-editing/crispr-rna-configurator/	-
8	CRISPRer	Web/online/graphic interface	http://jstacs.de/index.php/CRISPRer	-
9	CRISPRTarget	Web/online/graphic interface	http://bioanalysis.otago.ac.nz/CRISPRTarget	15
10	CRISPRfinder	Web/online/graphic interface	http://crispr.u-psud.fr/Server/	16
11	CRISPR gRNA Design tool	Web/online/graphic interface	https://www.dna20.com/eCommerce/cas9/input	-
12	COD (Cas9 Online Designer)	Web/online/graphic interface	http://cas9.wicp.net/	-
13	CRISPR	Web/online/graphic interface	http://crispr.mit.edu/	17
14	Cas9 design	Web/online/graphic interface	http://cas9.cbi.pku.edu.cn/	18
15	sgRNA Designer	Web/online/graphic interface	http://www.broadinstitute.org/rnai/public/ analysis-tools/sgrna-design	19
16	CRISPR-GA	Web/online/graphic interface	http://crispr-ga.net	20
17	Microhomology- Predictor	Web/online/graphic interface	http://www.rgenome.net/mich-calculator/	21
18	CRISPResso	Web/online/graphic interface	http://crispresso.rocks/	22
19	MAGeCK-VISPR	Web/online/graphic interface	https://bitbucket.org/liulab/mageck-vispr/	23
20	CrispRVariants	Web/online/graphic interface	https://github.com/markrobinsonuzh/CrispRVariants	24
21	Cas-Designer	Web server	http://www.rgenome.net/cas-designer/	25,26
22	CRISPR-ERA	Web server	http://CRISPR-ERA.stanford.edu.	27
23	CGAT	Web server	http://cbc.gdcb.iastate.edu/cgat/	28
24	E-CRISP	Web server	http://www.e-crisp.org/E-CRISP/	29
25	Cas-OFFinder	Web server	http://www.rgenome.net/cas-offinder/	30
26	CRISPRmap	Web server	http://rna.informatik.uni-freiburg.de/CRISPRmap/Input.jsp	31
27	CHOPCHOP	Web server	https://chopchop.rc.fas.harvard.edu/	32
28	CRISPR design tool	Web server	https://www.atum.bio/eCommerce/cas9/input	33
29	CropIT	Web server	http://cheetah.bioch.virginia.edu/AdliLab/CROP-IT/homepage.html	34
30	CasFinder	Web server	http://arep.med.harvard.edu/CasFinder/	35
31	ZiFiT	Web server	http://zifit.partners.org/ZiFiT/	36
32	CasOT	Web server	http://eendb.zfgenetics.org/casot/	37
33	Cas9-Design	Web server	http://cas9.cbi.pku.edu.cn/	38
34	CCTop	Web server	http://crispr.cos.uni-heidelberg.de/	39

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Table Continued....

S. no	Tool name	Standalone/ Web server	Link	Reference
35	COSMID	Web server	https://crispr.bme.gatech.edu/	40
36	sgRNA Scorer	Web server	https://crispr.med.harvard.edu/sgRNAScorer/	41
37	EuPaGDT	Web server	http://grna.ctegd.uga.edu/	42
38	CRISPR-RT	Web server	http://bioinfolab.miamioh.edu/CRISPR-RT/	43
39	BreakingCas	Web server	http://bioinfogp.cnb.csic.es/tools/breakingcas/index.php	44
40	CRISPR-DO	Web server	http://cistrome.org/crispr/	45
41	CRISPR/Cas9 target online predictor	Web server	http://crispr.cos.uni-heidelberg.de/	46
42	ProtospacerWB	Web server	http://www.protospacer.com/	47
43	CRISPR-P	Web server	http://cbi.hzau.edu.cn/crispr/	48
44	CRISPy	Web server	http://staff.biosustain.dtu.dk/laeb/crispy/	49
45	CLD	Web server	https://github.com/boutroslab/cld	50
46	CRISPOR	Web server	http://crispor.tefor.net/	51
47	Off-Spotter	Web server	https://cm.jefferson.edu/Off-Spotter/	52
48	SSC	Web server	http://crispr.dfci.harvard.edu/SSC/	53
49	CT-Finder	webserver	http://bioinfolab.miamioh.edu/ct-finder/	54
50	CRISPETa	Web server	http://crispeta.crg.eu/	55
51	WU-CRISPR	Web server	http://crispr.wustl.edu/	56
52	CRISPRscan	Web server	http://www.crisprscan.org/	57
53	CRISPR-ERA	Web server	http://crispr-era.stanford.edu/	58
54	Azimuth	Web server	https://www.microsoft.com/en-us/research/project/azimuth/	59
55	SSFinder	Web server	https://github.com/alaindomissy/ssfinder	60
56	CRISPR multitargeter	Web server	http://www.multicrispr.net/	61
57	flyCRISPR	Web server	http://tools.flycrispr.molbio.wisc.edu/targetFinder/	62
58	sgRNAcas9	Web server	http://www.biootools.com/col.jsp?id=103	63
59	Benchling	Web server	https://benchling.com/	--
60	AGEseq	Web server	http://aspendb.uga.edu:8085/	64
61	MAGeCK	Web server	https://sourceforge.net/p/mageck/wiki/Home/	65
62	Jack Lin's CRISPR/Cas9 gRNA finder	Web server	http://spot.colorado.edu/ _slin/cas9.html	66
63	GT-Scan	Web server	http://gt-scan.braembl.org.au/gt-scan/	67
64	PhytoCRISP-Ex	Web server, standalone	http://www.phytocrispex.biologie.ens.fr/CRISP-Ex/	68
65	CRISPR design	Web server	http://crispr.mit.edu/	-

Table 2 List of databases for designing sgRNA sequences

S. no	Tool name	Link	Reference
1	WGE	http://www.sanger.ac.uk/htgt/wge/	69
2	Cas-Database	http://www.rgenome.net/cas-database/	70
3	CrisprGE	http://crdd.osdd.net/servers/crisprge/.	71
4	CRISPRdb	http://crispr.u-psud.fr/crispr	72
5	COSMID	https://crispr.bme.gatech.edu/	73

Databases for designing CRISPR/Cas9 genome editing

Almost of the tools listed in Table 1 most likely have an inbuilt database for designing sgRNA sequences. However, for the sake of simplicity, we have listed 5 resources as "databases", which are have collated the sgRNA sequences and they can be easily accessed. We have included COSMID in both web server and database as it is an advanced resource available for users.[74]

Discussion

Microbes are the cell factories for the production of therapeutic and industrial biological products. Until recently, metabolic engineering

is at the forefront as a paradigm changing research area by which genomes can edit as desired. Nowadays, CRISPR and its associated proteins (Cas) are currently popular for precision genome editing in many organisms. Based on the global market statistics, the genome editing market is expected to touch around USD 5.54 Billion by the end of 2021 with a CAGR of 14.3%. Among different genome editing technologies the CRISPR segment stands alone with the largest share of the global genome editing market due to the ease usage of the technology.[75]

Conclusion

In this mini-review, we have highlighted an updated and currently accessible compilation of software (standalone and web server) and databases that are used for CRISPR/Cas9 genome editing method. With the availability of multiple resources for sgRNA design, the prediction accuracy and validated benchmark sets are utmost important for efficient targeted genome editing research.

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Conflict of interest

The author declares no conflict of interest.

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