

**Supplementary Table 1.** The most popular guide RNA design tools and their main features.

Tool	Input	Enzyme	Organism	On-Target Scoring Method	Off-Target Scoring Method	Features	Reference
CHOPCHOP	GeneID Coordinates Sequence	SpCas9; SpCas9n; Cas12a (Cpf1); CasX; Cas13 (C2C2); TALEN	Variety	Doench et al. 2014; Doench et al. 2016; Chari et al. 2015; Xu et al. 2015; Moreno-Mateos et al. 2015; G20	MIT specificity score; Cong et al., 2013	Designs primers for the edited site amplification; restriction sites map; exon-intron map; Integrates Shen et al. 2018 predictions of repair profile	[1,2]
CRISPOR	Coordinates Sequence	SpCas9; SpCas9- HF1; eSpCas9 1.1; ScCas9; iSpyMacCas9; SaCas9; xCas9; SaCas9-KKH; SpCas9-VQR; NmeCas9; SpCas9-VRER; StCas9; CjCas9; AsCas12a (Cpf1); LbCas12a (Cpf1)	Variety	Doench et al. 2016 Chari et al. 2015; Xu et al. 2015; Wu-Crisp Doench et al. 2014; Wang et al. 2014 Moreno-Mateos et al. 2015; Azimuth in-vitro crisprRank	MIT Specificity Score; CFD Specificity score	Designs primers for the edited site amplification; restriction sites map; provides sequences for in vitro expression or cloning of designed sgRNAs; Integrates Bae et al. 2014 predictions of repair profile and Chen et al. 2018 frameshift prediction	[3]
CRISPRscan	GeneID Sequence	SpCas9; AsCas12a (Cpf1); LbCas12a (Cpf1)	Variety	Moreno-Mateos et al. 2015;	MIT Specificity Score; CFD Specificity score; Cong et al., 2013	Appropriate for in- vitro guide RNA transcription under T7/Sp6 promoter; includes genetic variation;	[4]
IDT	GeneID Sequence	SpCas9	<i>Homo sapiens</i> <i>Mus musculus</i> <i>Rattus</i> <i>norvegicus</i> <i>Danio rerio</i> <i>Caenorhabditis</i> <i>elegans</i>	Own	Own	Offers pre-designed guide RNAs, design of custom guide RNAs and checking of designed guide RNAs	[5]
WGE	GeneID Sequence	SpCas9	<i>Homo sapiens</i> <i>Mus musculus</i>	Hodgkins et al. 2015	Hodgkins et al. 2015	Integrates Geniverse genome browser; exon- intron map; protein map; includes genetic variation; integrates Allen et al. 2019 predictions of repair profile	[6]
Benchling	GeneID Coordinates Sequence	SpCas9; NmeCas9; StCas9; TdCas9; SaCas9; AsCas12a (Cpf1); LbCas12a (Cpf1);	Variety	Doench et al. 2014; Doench et al. 2016;	MIT Specificity Score	Allows design of guides for base editing; restriction sites map; exon- intron map; protein map	[7]
GPP sgRNA Designer (CRISPick)	GeneID Sequence	SpCas9; SaCas9; AsCas12a (Cpf1); enCas12a (Cpf1)	<i>Homo sapiens</i> <i>Mus musculus</i> <i>Rattus</i> <i>norvegicus</i>	Doench et al. 2016;	CFD Specificity score	Includes genetic variation	[8–10]
CROP-IT	Coordinate Sequence	SpCas9	<i>Homo sapiens</i> <i>Mus musculus</i>	Singh et al. 2015	Singh et al. 2015	Incorporates chromatin state information	[11]
CRISTA	Coordinate Sequence	SpCas9	Variety	Abadi et al. 2017	BWA; Abadi et al. 2017	Incorporates advanced parameters in the prediction: genomic context, RNA	[12]

						thermodynamics, occurrence of bulges	
E-CRISP	GeneID Sequence	SpCas9	Variety	Heighwer et al. 2014; Doench et al. 2014; Xu et al. 2015	Bowtie2	Includes genetic variation	[13]
sgRNA Scorer 2.0	Sequence	SpCas9; SaCas9; AsCas12a (Cpf1); NmeCas9; StCas9	Variety	Chari et al., 2015; Chari et al., 2017	Aach et al. 2014		[14,15]
CasFinder	Coordinate Sequence	SpCas9; StCas9; NmeCas9	<i>Homo sapiens</i> <i>Mus musculus</i>	-	Aach et al. 2014	Exome-wide catalog of Cas9 cleavage sites	[16]
CCTop	Sequence	SpCas9; SpCas9-VQR; SpCas9-VRER; AsCas12a (Cpf1); LbCas12a (Cpf1); FnCas12a (Cpf1); SaCas9; StCas9; NmeCas9; TdCas9	Variety	CRISPRater	Stemmer et al. 2017	Includes genetic variation	[17,18]
DeepCRISPR	Sequence	SpCas9	<i>Homo sapiens</i>	Chuai et al. 2018	Chuai et al. 2018	Integrates the epigenetic information in different cell types	[19]
SNP-CRISPR	csv file containing genomic coordinates and variants	SpCas9	<i>Homo sapiens</i> <i>Mus musculus</i> <i>Danio rerio</i> <i>Drosophila melanogaster</i> <i>Rattus norvegicus</i>	Housden et al. 2015	BLAST	Dedicated to variant-specific CRISPR-Cas9 edits	[20,21]
AlleleAnalyzer	BCF/VCF file with genotypes	SpCas9; SaCas9	<i>Homo sapiens</i>	Keough et al. 2019	CRISPOR	An open-source Python software tool; dedicated to variant-specific CRISPR-Cas9 edits	[22]

AsCas12a—*Acidaminococcus* spp. Cas2a; CjCas9—*Campylobacter jejuni* Cas9; eSpCas9 1.1—enhanced specificity *Streptococcus pyogenes* Cas9 1.1; iSpyMacCas9—increased *Streptococcus pyogenes* and *Streptococcus macacae* Cas9; LbCas12a—*Lachnospiraceae* spp. Cas12a; NmeCas9—*Neisseria meningitidis* Cas9; SaCas9—*Staphylococcus aureus* Cas9; SaCas9-KKH—*Staphylococcus aureus* Cas9 KKH mutant; ScCas9—*Streptococcus canis* Cas9; SpCas9—*Streptococcus pyogenes* Cas9; SpCas9-HF1—*Streptococcus pyogenes* Cas9 High Fidelity 1; SpCas9n—*Streptococcus pyogenes* Cas9 nickase mutant; SpCas9-VQR—*Streptococcus pyogenes* Cas9 VQR mutant; SpCas9-VRER—*Streptococcus pyogenes* Cas9 VRER mutant; StCas9—*Streptococcus thermophilus* Cas9; TALEN—Transcription Activator-Like Effector Nuclease; TdCas9—*Treponema denticola* Cas9; xCas9—expanded PAM SpCas9.

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