**The CRISPR-Cas mechanism for adaptive immunity and alternate bacterial functions fuels diverse biotechnologies**

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Supplementary Table 1

**Supplementary Table 1. Expanded CRISPR-Cas biotechnology applications**

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| **Biotechnology Application** | **Cas Protein and CRISPR-Cas type, relevant activities, modifications** | **References** |
| A. Gene editing | 1a. SpyCas9 (type II-A), sequence specific DNA cleavage producing a mixture of staggered and blunt ends  1b. Hyper-accurate Cas9 (HypaCas9), four amino acid mutations near the RNA-DNA interface  1c. High-fidelity SpyCas9 (SpCas9-HF), mutation of four amino acids that non-specifically contact target DNA  1d. Enhanced specificity SpyCas9 (eSpCas9), three amino acid mutations in a groove that stabilizes the non-target strand of the target DNA  1e. Light activated Cas9, split into two fragments and fused to dimerization domains  1f. sgRNA regulation to control Cas9  1g. chemically inducible Cas9, various modifications  2. Cas12a (type V-A), sequence specific DNA cleavage producing staggered ends  3. CASCADE (type 1-E), sequence specific DNA binding, fused to FokI nuclease  4. CASCADE and Cas3 (type I-A), sequence specific DNA cleavage and degradation of one of the strands of a DNA  5. Cmr (type III-B), sequence specific interference of transcriptionally active DNA  6. Direct injection of CRISPR-Cas-based drug into the retina to treat hereditary blindness  7. Cas10-Csm and Csm6 (type III-A), viral DNA and RNA cleavage that increases viral gene editing efficiency by selecting against unedited phage  8. Anti-CRISPR proteins, CRISPR-Cas inhibition as a selectable marker for editing viral genomes  9. Anti-CRISPR proteins, CRISPR-Cas inhibition quenches gene editing reactions to minimize off-targeting | 1a. (Jinek et al., 2012; Cho et al., 2013; Jiang et al., 2013; Chu et al., 2015)  1b. (Chen et al., 2017)  1c. (Kleinstiver et al., 2016)  1d. (Slaymaker et al., 2016)  1e. (Nihongaki et al., 2015; Yu et al., 2020)  1f. (Lee et al., 2016; Liu et al., 2016b; Ferry et al., 2017; Maji et al., 2017; Tang et al., 2017)  1g. (Davis et al., 2015; Dow et al., 2015; Zetsche et al., 2015; Liu et al., 2016a; Maji et al., 2017)  2. (Hur et al., 2016; Ferenczi et al., 2017; Yan et al., 2017)  3. (Cameron et al., 2019)  4 & 5. (Li et al., 2015)  6. ClinicalTrials.gov Identifier: NCT03872479  7. (Nayeemul Bari and Hatoum-Aslan, 2019)  8. (Mayo-Muñoz et al., 2018)  9. (Marino et al., 2020) |
| B. Base editing | 1. SpyCas9 nickase (type II-A), sequence specific DNA binding, fused to both cytidine deaminase and uracil DNA glycosylase inhibitor  2. SpyCas9 (type II-A), sequence specific DNA binding, nuclease inactivated and fused to an engineered adenosine to inosine deaminase capable of targeting DNA  3. nuclease inactivated Cas12a (type V-A), sequence specific DNA binding, fused to cytidine deaminase and uracil DNA glycosylase inhibitor  4. nuclease inactivated Cas13 (type VI-B), sequence specific RNA binding, fused to adenosine deaminase | 1. (Komor et al., 2016)  2. (Gaudelli et al., 2017)  3. (Li et al., 2018)  4. (Cox et al., 2017) |
| C. Gene knockdown via ssRNA cleavage | 1. *Staphylococcus aureus* Cas9 (II-A), *Neisseria meningitidis* Cas9 (II-C) ssRNA cleavage  2. Cmr (III-B), ssRNA cleavage  3. Cas13 (VI-A and D), crRNA maturation and ssRNA cleavage  4. nuclease inactivated SpyCas9 (type II-A) fused to RNA endonuclease domain, sequence specific ssRNA binding | 1. (Rousseau et al., 2018; Strutt et al., 2018)  2. (Zebec et al., 2014)  3. (Abudayyeh et al., 2017; Konermann et al., 2018)  4. (Batra et al., 2017) |
| D. Transcriptional repression | 1. nuclease inactivated SpyCas9 (type II-A), sequence specific DNA binding  2. nuclease inactivated SpyCas9 (type II-A) fused to an epitope tag that recruits antibody-fused methyltransferase that methylates the genomic target to repress transcription, sequence specific DNA binding  3. nuclease inactivated Cas12a (type V-A), crRNA maturation and sequence specific DNA binding  4. CASCADE (type I-E), DNA binding | 1. (Qi et al., 2013)  2. (Huang et al., 2017)  3. (Zhang et al., 2017)  4. (Rath et al., 2014) |
| E. Gene activation | 1a. nuclease inactivated SpyCas9 (type II-A) fused to a transactivation domain, sequence specific DNA binding  1b. Light activated SpyCas9, nuclease inactivated and fused to a light-inducible heterodimerizing protein  2. Cas12a (type V-A), crRNA maturation and sequence specific DNA binding, fused to a transactivation domain  3. CASCADE (type I-E), crRNA maturation and sequence specific DNA binding, fused to plant transcriptional activation domain | 1a. (Perez-Pinera et al., 2013)  1b. (Polstein and Gersbach, 2015)  2. (Breinig et al., 2019)  3. (Young et al., 2019) |
| F. Nucleic acid detection | 1. Cas12a (type V-A), collateral ssDNA cleavage activity upon target binding  2. Cas13 (type VI-A and B), collateral ssRNA cleavage activity upon target binding  3. Csm6 (type III-A), non-specific nuclease | 1-3. (Gootenberg et al., 2018)  1. (Chen et al., 2018) |
| G. Antimicrobial | 1. SpyCas9 (type II-A), dsDNA interference  2. CASCADE and Cas3 (type I-E), dsDNA interference  3. Cas13a (type VI-A), promiscuous ssRNA cleavage  4. Anti-CRISPR proteins, CRISPR-Cas inhibition to expand the host range for phage-derived therapeutics | 1. (Citorik et al., 2014)  2. (Gomaa et al., 2014)  3. (Kiga et al., 2020)  4. (Marino et al., 2020) |
| H. Transposition or genome tagging | 1. CASCADE (type I-F), sequence specific DNA binding, fused to transposition protein  2. Cas1 and Cas2 (type I-E), sequence-selective DNA integration | 1. (Klompe et al., 2019)  2. (Wright et al., 2017) |
| I. Protect plants from both RNA and DNA viruses | 1. Cas13 (type VI-A), crRNA maturation and sequence specific ssRNA cleavage  2. SpyCas9 (type II-A), sequence specific DNA interference | 1. (Aman et al., 2018)  2. (Ali et al., 2015) |
| J. Decrease bacterial pathogenicity | Anti-CRISPR proteins to inhibit CRISPR-Cas systems involved in bacterial pathogenicity | (Marino et al., 2020) |
| K. Record transcription | Cas1:Cas2 complex (type III-D), natural fusion to reverse transcriptase, reverse transcription and sequence selective DNA integration | (Schmidt et al., 2018) |
| L. Store digital information | Cas1:Cas2 complex (type I-E), sequence selective DNA integration | (Shipman et al., 2017) |
| M. Visualizing nucleic acid localization | 1. nuclease inactivated SpyCas9 (type II-A) fused to GFP, sequence specific binding to target DNA  2. nuclease inactivated Cas13 (type VI-A) fused to GFP, sequence specific ssRNA binding | 1. (Chen et al., 2013; Nelles et al., 2016)  2. (Abudayyeh et al., 2017) |
| N. Mediate chromatin immunoprecipitation for downstream analysis of epiproteome (interacting proteins, histone post translational modifications, RNAs, and neighboring genomic regions) via mass spectrometry and nucleic acid sequencing | nuclease inactivated and affinity tagged SpyCas9 (type II-A), sequence specific DNA binding | (Waldrip et al., 2014; Fujita and Fujii, 2015) |
| O. Subnuclear proteomic profiling | nuclease inactivated SpyCas9 (type II-A) fused to APEX2 (an engineered soybean ascorbate peroxidase that labels near-by proteins with biotin), sequence specific DNA binding | (Gao et al., 2019) |
| P. Influence mRNA splicing outcomes without hindering translation to protein (potential treatment for diseases caused by gene mis-splicing) | Cas13d (CasRx, type VI-D), sequence specific DNA binding, nuclease inactivated | (Konermann et al., 2018) |

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