GAVIN PUBLISHERS

Biomarkers & Applications

Sampathi S. Biomark Applic: BMAP-111.

DOI: 10.29011/BMAP-111. 100111

Short Commentary

CRISPR-Cas9 Mediated Gene Editing: A Revolution in Genome Engineering

Shilpa Sampathi*

Department of Biochemistry, Vanderbilt University Medical Center, USA

***Corresponding author:** Shilpa Sampathi, Department of Biochemistry, Vanderbilt University Medical Center, Nashville, TN 37232, USA. Tel: +16159363584; Email: shilpa.sampathi@vanderbilt.edu

Citation: Sampathi S (2017) CRISPR-Cas9 Mediated Gene Editing: A Revolution in Genome Engineering. Biomark Applic: BMAP-111. DOI: 10.29011/BMAP-111. 100111

Received Date: 28 August, 2017; **Accepted Date:** 7 September, 2017; **Published Date:** 12 September, 2017

Commentary

CRISPR-Cas technology has taken over the laboratories around the world as a revolution. This genome engineering technology is powerful in that it provides a robust method of systematically analyzing gene functions, disease target screening and validation, gene knockout studies for factors contributing to tumor progression, genome imaging, screening etc. Its vast applications are only emerging and in the future, hold a great promise towards better understanding of disease progression and accelerate the cure.

CRISPR-Cas9 systems emerged from bacteria and archaea where this mechanism is used for conferring adaptive immunity against bacteriophages and plasmids [1,2]. CRISPR stands for "Clustered regularly interspersed short palindromic repeats". Its associated protein Cas9 is an endonuclease that uses a RNA duplex of tracr RNA: cr RNA (trans-activating cr RNA and CRISPR targeting RNA) as a guide to form base pairs with the target DNA sequences thus allowing Cas9 to make a site-specific Double Stranded Breaks (DSB). This dual tracr RNA: cr RNA can be engineered as a single guide RNA (sgRNA) sequence that has a 20nt at the 5' end of the sgRNA which is required for determining the target site and a duplex RNA structure at the 3' end that binds to the Cas9. This design allows changes to be made at any target DNA sequence by changing the sequences in the guide RNA that can base pair with the target DNA. Hence this technology is a powerful genome engineering tool allowing sequence specific gene editing [3-5].

DNA target recognition in the CRISPR-Cas system requires a short sequence site known as the proto spacer adjacent motif (PAM) that is adjacent to the target sequence on the DNA of interest. The most commonly used Cas9 endonucleases is adapted from *Streptococcus pyogenes* and it has been optimized for its use in gene editing. The Cas9 has been engineered to derive variants that can increase specificity and cleaving and can be used for wide variety of applications [6].

Cas9 has a HNH nuclease domain that is required to cleave the target strand and RuvC-like nuclease domain that cleaves the non-target strand. By mutating any one of the two domains results in a nickase Cas9 (nCas9) that can now only cleave one strand. This feature is being explored to increase the specificity and cleaving ability. By mutating both the domains of Cas9, an endonuclease which retains the RNA guided DNA binding ability but has lost the cleaving ability is generated. This form of Cas9 called the dCas9 can be used in epigenetic regulation studies where it can be used to various effects or to mediate site-specific regulation without any target DNA cleaving [7-9].

CRISPR-Cas systems have found applications in high-throughput screening, disease target screening and validation, genome imaging etc. [10-20]. The future holds a great promise in deciphering processes that were previously elusive in pathways of cancer and in disease pathogenesis.

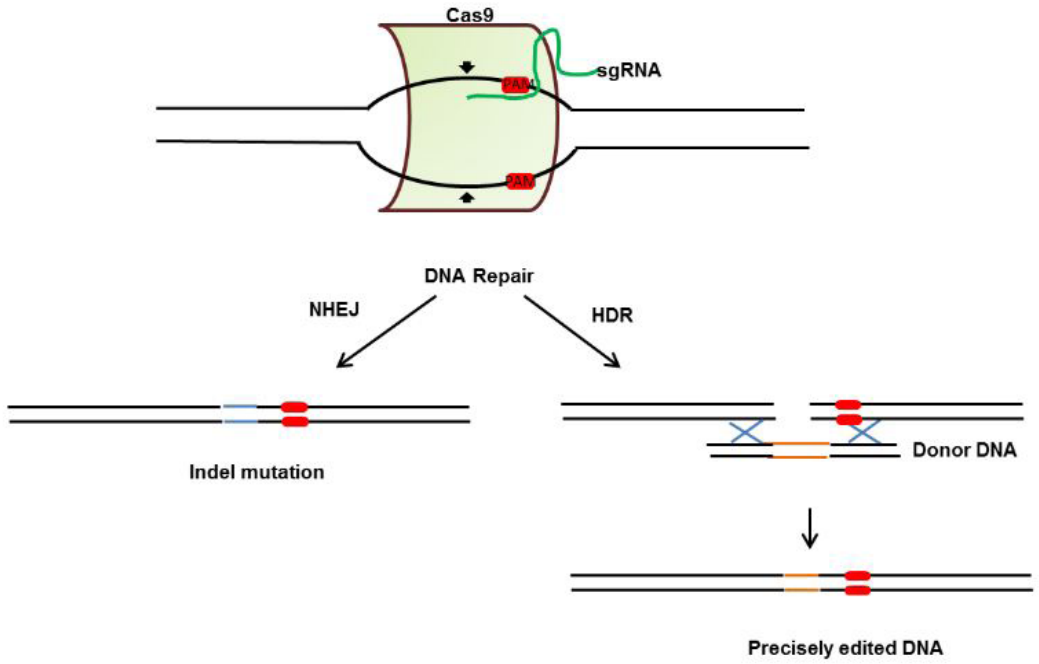

Figure: Genome editing by CRISPR-Cas9 RNA guided Cas9 is an endonuclease that is directed to the target DNA and creates a site-specific double stranded break that is then repaired by either Non-Homologous End Joining (NHEJ) that can create Indels or Homology Directed Repair (HDR). In the latter type, a donor DNA oligo can be used to precisely edit the DNA.

References

1. Horvath P, Barrangou R (2010) CRISPR/Cas, the immune system of bacteria and archaea. Science 327:167-170.
2. Garneau JE, Dupuis MÈ, Villion M, Romero DA, Barrangou R, et al. (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophages and plasmid DNA. Nature 468: 67-71.
3. Le Cong F, Ann Ran, David Cox, Shuailiang Lin, Robert Barretto, et al. (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339: 819-823.
4. Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA (2013) RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nat Biotechnol 31: 233-239.
5. Yang H, Wang H, Shivalila CS, Cheng AW, Shi L, et al. (2013) One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. Cell 154: 1370-1379.
6. Sander JD, Joung JK (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32: 347-355.
7. Mali P, Esvelt KM, Church GM (2013) Cas9 as a versatile tool for engineering biology. Nat Methods10: 957-963.
8. Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, et al. (2013) DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol 31: 827-832.
9. Ran FA, Hsu PD, Lin CY, Gootenberg JS, Konermann S, et al. (2013) Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell 154: 1380-1389.
10. Kim JM, Kim D, Kim S, Kim JS (2014) Genotyping with CRISPR-Cas-derived RNA-guided endonucleases. Nat Commun 5: 3157.
11. Lokody I (2014) Genetic therapies: Correcting genetic defects with CRISPR-Cas9. Nat Rev Genet 15: 63.
12. Vikram Pattanayak, Steven Lin, John P Guilinger, Enbo Ma, Jennifer A Doudna, et al. (2013) High-throughput profiling of off-target DNA cleavage reveals RNA-programmed Cas9 nuclease specificity. Nat Biotechnol 31: 839-843.
13. Abba Malina, John R Mills, Regina Cencic, Yifei Yan, James Fraser, et al. (2013) Repurposing CRISPR/Cas9 for in situ functional assays. Genes Dev 27: 2602-2614.
14. Xiao A, Wang Z, Hu Y, Wu Y, Luo Z, et al. (2013) Chromosomal deletions and inversions mediated by TALENs and CRISPR/Cas in zebrafish. Nucleic Acids Res 41: e141.
15. Amitai G, Sorek R (2017) Intracellular signaling in CRISPR-Cas defense. Science 357: 550-551.
16. Bolukbasi MF, Gupta A, Wolfe SA (2016) Creating and evaluating accurate CRISPR-Cas9 scalpels for genomic surgery. Nat Methods 13: 41-50.
17. Auer TO, Duroure K, De Cian A, Concordet JP, Del Bene F (2014) Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. Genome Res 24: 142-153.
18. Deans RM, Morgens DW, Ökesli A, Pillay S, Horlbeck MA, et al. (2016) Parallel shRNA and CRISPR-Cas9 screens enable antiviral drug target identification. Nat Chem Biol 12: 361-366.
19. Du D, Qi LS (2016) CRISPR Technology for Genome Activation and Repression in Mammalian Cells. Cold Spring Harb Protoc 2016: 1.
20. Guo Y, Xu Q, Canzio D, Shou J, Li J, et al. (2015) CRISPR Inversion of CTCF Sites Alters Genome Topology and Enhancer/Promoter Function. Cell 162: 900-910.