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CRISPR/CAS9 IN GENOME EDITING: A NATURE GIFTED MOLECULAR TOOL

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Abstract: *The Cas9 protein derived from type II CRISPR as a part of bacterial immune system has been raising up as a useful genetic tool for genomic engineering in various life forms. As RNA-guided DNA endonucleases, the Cas9 could be effectively customized to marked new DNA sequence sites by adjusting guided RNA sequences; it has been appeared as new emerging DNA editing technology. The nuclease-disable types of Cas9 has provided adaptable RNA guided DNA focusing on regulation and visualization of genomic DNA, just as for restoring the epigenetic forms and status, all has been shown in a an accurate sequence. Through these proceed; the researchers have started to explore conceivable uses of Cas9 in medical, agriculture, pharmaceutical and livestock sciences.*

Keywords: Cas9/CRISPR, endonucleases, RNA, medical, agriculture, pharmaceutical, livestock

Introduction

While approach of reliable opinion from molecular science, researchers had tried to create new innovations to adjust or hold the genomics (Hunter et al., 2017; Thornton et al., 2018). Exact altering and guideline of genomic data is fundamental to understand gene action in sense of regulation and expression (Abudayyeh et al., 2017; Cong et al., 2013). Amid the previous decade, mechanical achievements have made genomics altering or guideline essentially less demanding. One ongoing innovation has adjusted the clustered regularly interspaced short palindromic repeats (CRISPR), Cas9 bacterial protein safe framework as a basic, RNA-guided stage for very productive and precise genomics altering and guideline in differing life forms, in this manner making progressive methods for biomedical research and new conceivable outcomes for treating hereditary scatters (Barrangou et al., 2007; Brouns et al., 2008). The maganucleases, or on the other hand homing nuclease, are among the main types of nucleases which were designed to mark precise genomic editing for a specific gene site. The meganucleases are the gathering of nucleases which perceive long chain of nuclease successions and insert a double standard break at their target sites (Jiang and Doudna, 2017; Ran et al., 2013; Terns and Terns, 2011). The long recognition sequence of maganucleases may happen just once inside a genome, which encouraged its utilization for site precise genome editing or altering. The maganucleases could be redesigned for the intention of novel sequence through methodologies, for example, protein building, molecular regulation, composition-based plan, despite the fact that the methodology is generally work concentrated (Jackson et al., 2017; Jiang and Doudna, 2017; Knott and Doudna, 2018). Different types of genome altering techniques are included zinc-finger nucleases (ZFNs) and the transcriptional activator-like effector nucleases (TALENs). Nonetheless, according to the fact that these tackles work through DNA-protein communications, focusing to a new site have need of designing and copying another protein, which blocks TALENs and ZFNs from being utilized for high throughout put purposes (Jiang and Doudna, 2017; Shalem et al., 2014). Rather than mainly known DNA restricting proteins, RNA guided nuclease is Cas9 protein whose sequences preciseness generally emerges from Watson–Crick model from which its guide RNA and objective DNA site. Just as different applications for making it a perfect stage for high-throughput sequencing, precise quality altering Cas9 could be customized to mark for new sites essentially by guided its guided RNA sequences (Hille et al., 2018; Jiang and Doudna, 2017). In the editing of the genomics in a wide scope of living beings regular endonuclease action have been co-settled on targeted sequences of parasites, plants, animals and microbes. Collections of effectors could be melded for repressors, transcriptional activators and epigenetic changer to empower series precise genomic guideline and nuclease disabled Cas9 have been built (Hess et al., 2017). Not with standing applications in genomics altering and guideline, to permit through imaging or visualizing of genomic area in living cells DNA-restricting proteins, for example TALEs, ZFs

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and deactivated Cas9, have been fused to fluorescent proteins (Chen et al., 2019a; Levin, 2019). Moreover, for examining proteins of deactivated Cas9 has likewise been utilized that interface with precise loci and it might conceivably be utilized to marked RNA. In this assessment, we represent the operational system of Cas9 dependent on the discoveries of essential or biochemical learning (Palermo et al., 2018; Ryan et al., 2018). The researchers have various utilizations of CRISPR in genome editings, guideline, furthermore, visualization in mammalian cells, featuring intensity of these novel frameworks in living organisms (Chew, 2018; Lee et al., 2018; Makarova et al., 2019).

CRISPR framework could shape the basis of an adaptable genomic building toolbox, along these lines several described Cas protein tie to nucleic acids, the Cas9, that cuts objected DNA in type 2 CRISPR system, is the mainly generally utilized for genome altering what's more, guideline among the Cas protein. Duplex of two RNAs guide the Cas9 marked cleavage: tracr RNA fused with cr RNA and this is unique to CRISPR type 2 systems and the cr RNA that identify the attacking DNA upto 20–base pair (bp) (Chen and Doudna, 2017; Dugar et al., 2018). For proficient genomic editing Cas9 conjugate with the tracrRNA-crRNA and could be re use. For gene editing and other applications the mainly used applications are single-RNA, single-protein and sgRNA-Cas9 (Koonin and Makarova, 2019; Li et al., 2018; Shmakov et al., 2018). Attaching of the Cas9–sgRNA composite encourage slash inside the base coupling section. Hence, Cas9 be able to remark fundamentally any genomic loci having a PAM (Protospacer-Adjacent Motif) sequences, basically by converting a roughly 20 base pairs, area of the sgRNA to couple with the DNA sequence of importance and making it an effortlessly grouping proposal for precise genomic editing and targeting (Lo and Qi, 2017; Meng et al., 2018; Stella et al., 2017).

Working Model for DNA cleavage and Guide RNA binding

Effective components of Cas9 have been representing through incorporating auxiliary examinations and in vitro examines. In this study, the Cas9 protein when not bound by sgRNA at a spot keeps up auto inhibited compliance, in which the dynamic locales in the HNH nuclease domains are hindered by the RuvC area (Chen et al., 2018; Wilson et al., 2018). For making a focal channel among the two projections for DNA by a sgRNA prompts a conformational change, in this manner going into a DNA detection-able position. By three-dimensional dissemination the subsequent Cas9 sgRNA premarking compound could study DNA for PAMs. Throughout its PI area the Cas9– sgRNA compound ties to a PAM, sgRNA– DNA heteroduplex arrangement encouraged by the neighborhood DNA filament division in the PAM proximal surroundings (Belotserkovskii et al., 2018; Mollanoori et al., 2018; Palermo et al., 2017). If a critical match present among the steer RNA portion and the objective DNA then Cas9– sgRNA complex will keep on loosening up the DNA. The solid lead RNA– mark DNA base matching associations further advance DNA twofold filament partition and RNA DNA multiduplex arrangement, which continues by the PAM-proximal district along with construct total R loop (Doxzen and Doudna, 2017; Gleditzsch et al., 2019; Hwang and Maxwell, 2019).

Mechanisms for Genome Editing

From the time when its discovery, for genome altering in numerous living beings Cas9 has been widely utilized. Cas9 is a programmable, arrangement explicit endonuclease, designed like ZFNs and TALENs. Like diverse nucleases, Cas9-intervened genome altering is accomplished by two-advance methods (Hwang and Maxwell, 2019; Zeng et al., 2018; Zuo and Liu, 2017). DNA cleavages pursued by DNA fix. For the creation of a double strand breaks (DSB), the sgRNA coordinates Cas9 to a particular genome area, where inborn cell components triggers DNA fix, for example Non-homologous end joining (NHEJ) or HDR. At the DSB spot no homologous end fusion cause's almainly unequal enclosure and erasure alteration, in this way may prompt worth knockout (Chen et al., 2019b; Xia et al., 2019). Next to the DSB position through homologous fusion guided by a giver DNA format homologous direct repair could be misused to create the ideal succession substitution, causing focused on quality editing, addition, mutagenesis, or quality amendment (Bhargava et al., 2018; Saglam-Metiner et al., 2019). In this way, the CRISPR/Cas9 framework gives an unbelievable phase for grouping explicit, including class knockout, genomics altering, class knock in, and genomics altering combination rectifications and mutagenesis.

To comprehend working of explicit Cas9-intervened gene altering framework has been comprehensively utilized to the rear hereditary traits, for illness displaying and irresistible maladies and also for showing new helpful conspires in various models of hereditary. By just making another sgRNA that sets with the ideal DNA focusing on location contiguous PAM which support to refocus Cas9 to another DNA site (Kosicki et al., 2017; Li et al., 2019). When each 8 base pairs inside the genome of Sp Cas9, where the 5'-NGG-3' PAM occurred, in this way permitting practically any editing occurred which has been focused on. The scopes of Cas9 mark able genomic arrangement extend by Cas9s from different variety which had distinctive PAMs of various dimension

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and involving an assortment of successions (Liu et al., 2019; Soyars et al., 2018). With modified PAM successions the building of existing Cas9s has additionally prompted the making of new forms of Cas9, along these lines extending the mark able cut inside the mammalian genome. From parasites and plants to an assortment of creatures the utilization of the Cas9 stage has extraordinarily expanded the proficiency of producing transgenic organisms (Hille et al., 2018). This innovation likewise makes it a lot less demanding to produce sickness models for hereditary disarranges and illnesses like cancer, which helps our comprehension of the molecular apparatus of these neurotic procedures. By presenting a few sgRNAs all the while Cas9 could be effectively modified to alter different genomic loci in the meantime (Chew, 2018; Dugar et al., 2018). This could be connected to create substantial scale chromosomal adjustments. For instance, a similar chromosome may create focused on erasures or reversals of the middle of the road section of DNA by making a couple of DSBs at close-by area inside, and through prompting a focused on chromosomal translocation, could make two DSBs in various chromosomes. These Cas9-intervened, directed improvements might be valuable for making disease models by imitating modifications that happen in human infection conditions (Doxzen and Doudna, 2017; Gleditzsch et al., 2019). The Cas9 structure likewise could possibly fix or treat numerous illnesses, including hereditary diseases, HIV, and malignant growth. Viral genomics could be clear and inactive, when Cas9 used to bring with contaminated cell collectively in sgRNAs focusing on essential viral genome components and along these lines, guards the cells or living being from diseases with Epstein– Barr infection, HIV, human papilloma virus and hepatitis B infection (Chen et al., 2019b; Jiang and Doudna, 2017). Besides, altering the qualities of HIV co-receptors by utilizing CRISPR-Cas9 or ZFNs in the host genome, which encodes co-receptors of HIV, makes cell protection from the HIV-1 infection and, in this manner, it may control disease. Moreover, numerous researchers have detailed utilizing the Cas9-interceded genome altering structure for rectifying infection related transformations in human immature microorganisms and germ line cells, as well as in creature substantial and actuated pluripotent undifferentiated cells (Hunter et al., 2017; Koonin and Makarova, 2019). An incomplete rundown incorporates the Dystrophic in Duchene strong dystrophy, Fah quality in genetic tyrosinemia, CFTR in cystic fibrosis, Crygc in waterfalls, HBB (gene which produce beta-globin) in β-thalassemia.

Fundamentally, for vast scale genome-wide knockout screens the Cas9 stage has been utilized that had been beforehand unviable. Beforehand, gene expression at RNA stage without influencing DNA arrangement by genome loss-of-work viewing depended on the RNA impedance loom. A little meddling RNA that base sets by its objective emissary RNA (mRNA) will prompt by diminishing in the security and interpretation of their objective, which is present in RNA impedance (Levin, 2019; Li et al., 2018). A counterfeit RNA particle containing a clip that is then handled into the develop little meddling RNA makeup by the cell's endogenous little RNA alleyway, the little intrusive RNA could be blended or delivered from a vector encoding a small clasp RNA (Jackson et al., 2017; Jiang and Doudna, 2017). Along these lines, utilizing a library of little meddling RNAs or small hairpin RNAs extensive scale gene knockdown screening could be accomplished. Similarly, researchers could develop the CRISPR-Cas9 stage to visualize genes participating to a biological method by generating a collection of sgRNAs marking gene coding area (Koonin and Makarova, 2019; Makarova et al., 2019). The interfering RNA technique may guide to incomplete gene suppression whereas, the Cas9–sgRNA come close to generates mutation at the marked loci and may reason of whole failure of gene purpose. Thus, when marked the same gene, a more pronounced phenotype produced by CRISPR/Cas9 than RNA interfering, which formulate recognition of applicable genes easier. One thoroughfare to validate the candidate genes recognized by the CRISPR/Cas9 move toward to re-express the marked gene. Likewise, hits exposed with the RNA interfering come within reach of may be validated by expression of an RNA interfering- opposing transcription (Palermo et al., 2018; Shalem et al., 2014). Not all axons contain such a mark able succession, whereas shRNA or siRNA collection, in standard, could end any mRNA progression in conditions of boundaries in marking, the CRISPR/Cas9 process could marked only a series neighboring to PAM (Li et al., 2018; Lo and Qi, 2017; Makarova et al., 2019).

Moreover, the use of the CRISPR/Cas9 achievement move toward to study essential genes is demanding, for the reason that deletion of basic genes causes a poisonous result that put off mainly useful assess. Both methods could shape the basis of a successful display, and the process of selection will depend on the needs of the research.

Conflict of interest

The authors have declared absence of any conflict of interest.

References

Abudayyeh, O. O., Gootenberg, J. S., Essletzbichler, P., Han, S., Joung, J., Belanto, J. J., Verdine, V., Cox, D. B., Kellner, M. J., and Regev, A. (2017).

RNA targeting with CRISPR–Cas13. *Nature* **550**, 280-284.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., and Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science* **315**, 1709-1712.

Belotserkovskii, B. P., Tornaletti, S., D'Souza, A. D., and Hanawalt, P. C. (2018). R-loop generation during transcription: Formation, processing and cellular outcomes. *DNA repair* **71**, 69-81.

Bhargava, R., Sandhu, M., Muk, S., Lee, G., Vaidehi, N., and Stark, J. M. (2018). C-NHEJ without indels is robust and requires synergistic function of distinct XLF domains. *Nature communications* **9**, 1-14.

Brouns, S. J., Jore, M. M., Lundgren, M., Westra, E. R., Slijkhuis, R. J., Snijders, A. P., Dickman, M. J., Makarova, K. S., Koonin, E. V., and Van Der Oost, J. (2008). Small CRISPR RNAs guide antiviral defense in prokaryotes. *Science* **321**, 960-964.

Chen, J. S., and Doudna, J. A. (2017). The chemistry of Cas9 and its CRISPR colleagues. *Nature Reviews Chemistry* **1**, 1-15.

Chen, K., Wang, Y., Zhang, R., Zhang, H., and Gao, C. (2019a). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual review of plant biology* **70**, 667-697.

Chen, M., Mao, A., Xu, M., Weng, Q., Mao, J., and Ji, J. (2019b). CRISPR-Cas9 for cancer therapy: Opportunities and challenges. *Cancer letters* **447**, 48-55.

Chen, Y.-C., Sheng, J., Trang, P., and Liu, F. (2018). Potential application of the CRISPR/Cas9 system against herpesvirus infections. *Viruses* **10**, 291.

Chew, W. L. (2018). Immunity to CRISPR Cas9 and Cas12a therapeutics. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine* **10**, e1408.

Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., and Marraffini, L. A. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819-823.

Doxzen, K. W., and Doudna, J. A. (2017). DNA recognition by an RNA-guided bacterial Argonaute. *PloS one* **12**, e0177097.

Dugar, G., Leenay, R. T., Eisenbart, S. K., Bischler, T., Aul, B. U., Beisel, C. L., and Sharma, C. M. (2018). CRISPR RNA-dependent binding and cleavage of endogenous RNAs by the Campylobacter jejuni Cas9. *Molecular cell* **69**, 893-905. e7.

Gleditzsch, D., Pausch, P., Müller-Esparza, H., Özcan, A., Guo, X., Bange, G., and Randau, L. (2019). PAM identification by CRISPR-Cas effector complexes: diversified mechanisms and structures. *RNA biology* **16**, 504-517.

Hess, G. T., Tycko, J., Yao, D., and Bassik, M. C. (2017). Methods and applications of CRISPR-mediated base editing in eukaryotic genomes. *Molecular cell* **68**, 26-43.

Hille, F., Richter, H., Wong, S. P., Bratovič, M., Ressel, S., and Charpentier, E. (2018). The biology of CRISPR-Cas: backward and forward. *Cell* **172**, 1239-1259.

Hunter, M. C., Smith, R. G., Schipanski, M. E., Atwood, L. W., and Mortensen, D. A. (2017). Agriculture in 2050: recalibrating targets for sustainable intensification. *Bioscience* **67**, 386-391.

Hwang, S., and Maxwell, K. L. (2019). Meet the anti-CRISPRs: Widespread protein inhibitors of CRISPR-Cas systems. *The CRISPR journal* **2**, 23-30.

Jackson, S. A., McKenzie, R. E., Fagerlund, R. D., Kieper, S. N., Fineran, P. C., and Brouns, S. J. (2017). CRISPR-Cas: Adapting to change. *Science* **356**.

Jiang, F., and Doudna, J. A. (2017). CRISPR–Cas9 structures and mechanisms. *Annual review of biophysics* **46**, 505-529.

Knott, G. J., and Doudna, J. A. (2018). CRISPR-Cas guides the future of genetic engineering. *Science* **361**, 866-869.

Koonin, E. V., and Makarova, K. S. (2019). Origins and evolution of CRISPR-Cas systems. *Philosophical Transactions of the Royal Society B* **374**, 20180087.

Kosicki, M., Rajan, S. S., Lorenzetti, F. C., Wandall, H. H., Narimatsu, Y., Metzakopian, E., and Bennett, E. P. (2017). Dynamics of indel profiles induced by various CRISPR/Cas9 delivery methods. *In* "Progress in Molecular Biology and Translational Science", Vol. 152, pp. 49-67. Elsevier.

Lee, Y. T., Tan, Y. J., and Oon, C. E. (2018). Molecular targeted therapy: treating cancer with specificity. *European journal of pharmacology* **834**, 188-196.

Levin, A. A. (2019). Treating disease at the RNA level with oligonucleotides. *New England Journal of Medicine* **380**, 57-70.

Li, K., Cai, D., Wang, Z., He, Z., and Chen, S. (2018). Development of an efficient genome editing tool in Bacillus licheniformis using CRISPR-Cas9 nickase. *Applied and Environmental Microbiology* **84**.

Li, R., Liu, C., Zhao, R., Wang, L., Chen, L., Yu, W., Zhang, S., Sheng, J., and Shen, L. (2019). CRISPR/Cas9-Mediated SlNPR1 mutagenesis

reduces tomato plant drought tolerance. *BMC plant biology* **19**, 1-13.

Liu, B., Saber, A., and Haisma, H. J. (2019). CRISPR/Cas9: a powerful tool for identification of new targets for cancer treatment. *Drug discovery today* **24**, 955-970.

Lo, A., and Qi, L. (2017). Genetic and epigenetic control of gene expression by CRISPR–Cas systems. *F1000Research* **6**.

Makarova, K. S., Wolf, Y. I., Iranzo, J., Shmakov, S. A., Alkhnbashi, O. S., Brouns, S. J., Charpentier, E., Cheng, D., Haft, D. H., and Horvath, P. (2019). Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. *Nature Reviews Microbiology*, 1-17.

Meng, X., Hu, X., Liu, Q., Song, X., Gao, C., Li, J., and Wang, K. (2018). Robust genome editing of CRISPR-Cas9 at NAG PAMs in rice. *Science China Life Sciences* **61**, 122-125.

Mollanoori, H., Shahraki, H., Rahmati, Y., and Teimourian, S. (2018). CRISPR/Cas9 and CAR-T cell, collaboration of two revolutionary technologies in cancer immunotherapy, an instruction for successful cancer treatment. *Human immunology* **79**, 876-882.

Palermo, G., Chen, J. S., Ricci, C. G., Rivalta, I., Jinek, M., Batista, V. S., Doudna, J. A., and McCammon, J. A. (2018). Key role of the REC lobe during CRISPR–Cas9 activation by 'sensing','regulating', and 'locking'the catalytic HNH domain. *Quarterly reviews of biophysics* **51**.

Palermo, G., Miao, Y., Walker, R. C., Jinek, M., and McCammon, J. A. (2017). CRISPR-Cas9 conformational activation as elucidated from enhanced molecular simulations. *Proceedings of the National Academy of Sciences* **114**, 7260-7265.

Ran, F. A., Hsu, P. D., Wright, J., Agarwala, V., Scott, D. A., and Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. *Nature protocols* **8**, 2281-2308.

Ryan, D. E., Taussig, D., Steinfeld, I., Phadnis, S. M., Lunstad, B. D., Singh, M., Vuong, X., Okochi, K. D., McCaffrey, R., and Olesiak, M. (2018). Improving CRISPR–Cas specificity with chemical modifications in single-guide RNAs. *Nucleic acids research* **46**, 792-803.

Saglam-Metiner, P., Gulce-Iz, S., and Biray-Avci, C. (2019). Bioengineering-inspired three-dimensional culture systems: Organoids to create tumor microenvironment. *Gene* **686**, 203-212.

Shalem, O., Sanjana, N. E., Hartenian, E., Shi, X., Scott, D. A., Mikkelsen, T. S., Heckl, D., Ebert, B. L., Root, D. E., and Doench, J. G. (2014). Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* **343**, 84-87.

Shmakov, S. A., Makarova, K. S., Wolf, Y. I., Severinov, K. V., and Koonin, E. V. (2018). Systematic prediction of genes functionally linked to CRISPR-Cas systems by gene neighborhood analysis. *Proceedings of the National Academy of Sciences* **115**, E5307-E5316.

Soyars, C. L., Peterson, B. A., Burr, C. A., and Nimchuk, Z. L. (2018). Cutting edge genetics: CRISPR/Cas9 editing of plant genomes. *Plant and Cell Physiology* **59**, 1608-1620.

Stella, S., Alcón, P., and Montoya, G. (2017). Class 2 CRISPR–Cas RNA-guided endonucleases: Swiss Army knives of genome editing. *Nature Structural & Molecular Biology* **24**, 882.

Terns, M. P., and Terns, R. M. (2011). CRISPR-based adaptive immune systems. *Current opinion in microbiology* **14**, 321-327.

Thornton, P. K., Whitbread, A., Baedeker, T., Cairns, J., Claessens, L., Baethgen, W., Bunn, C., Friedmann, M., Giller, K. E., and Herrero, M. (2018). A framework for priority-setting in climate smart agriculture research. *Agricultural Systems* **167**, 161-175.

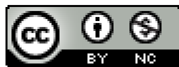
Wilson, C. J., Fennell, T., Bothmer, A., Maeder, M. L., Reyon, D., Cotta-Ramusino, C., Fernandez, C. A., Marco, E., Barrera, L. A., and Jayaram, H. (2018). Response to "Unexpected mutations after CRISPR–Cas9 editing in vivo". *Nature methods* **15**, 236-237.

Xia, A.-L., He, Q.-F., Wang, J.-C., Zhu, J., Sha, Y.-Q., Sun, B., and Lu, X.-J. (2019). Applications and advances of CRISPR-Cas9 in cancer immunotherapy. *Journal of medical genetics* **56**, 4-9.

Zeng, Y., Cui, Y., Zhang, Y., Zhang, Y., Liang, M., Chen, H., Lan, J., Song, G., and Lou, J. (2018). The initiation, propagation and dynamics of CRISPR-SpyCas9 R-loop complex. *Nucleic acids research* **46**, 350-361.

Zuo, Z., and Liu, J. (2017). Structure and dynamics of Cas9 HNH domain catalytic state. *Scientific reports* **7**, 1-13.

[Citation: Bashir, A.M., Ali, Q., Rashid M.S., Malik, A. (2020). CSRISPR-Cas9 in genome editing: A nature gifted molecular tool. *Biol. Clin. Sci. Res. J.,* **2020***:* 18 doi: https://doi.org/10.54112/bcsrj.v2020i1.18]



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