STM JOURNALS

Research & Reviews: Journal of Computational Biology
ISSN: 2319-3433 (Online), ISSN: 2349-3720 (Print)
Volume 7, Issue 3
www.stmjournals.com

Studies of CRISPR/CAS 9 Gene Interactions in *Streptococcus pyogenes*

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Abstract

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/cas9/single guide RNA (sgRNA) system is a DNA editing system in mostly studied in prokaryotes. Studies of CRISPR/cas9 gene interactions in Streptococcus pyogenes has been conducted using in silico approaches in the present research. Proteins like DNA polymerase I (polA), NAD-dependent DNA ligase LigA (ligA), Translaldolase (tal), Thymidine kinase (tdk), Streptococcal exotoxin I (speI), Phage associated protein (SPy_0679) and Hypothetical proteins like SPy_1047, SPy_1048, csn2 and SPy_2034 has shown interaction with cas9. The streptococcus pyogenes has shown relevance with Microbacterium arborescens and Klebsiella pneumonia based on cas9 protein sequence homology. Further in vitro and in vivo research has to be conducted for better understanding to propose the mechanism.

Keywords: *CRISPR, Cas9, Streptococcus pyogenes*

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INTRODUCTION

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/cas9/single guide RNA (sgRNA) system is a DNA editing system in bacteria, yeast, zebrafish, fruit fly and human cells [1]. In the present decades, advances in medical and scientific research for guiding synthetic transcription factors using DNA-binding proteins plays an important role in development of novel medicine and biotechnology [2]. The resistance to bacteriophages with the integrated new spacers of genomes in most Bacteria and Archaea are due to presence of CRISPR with associated cas genes [3]. A multiplexed genome editing with disruption of five genes (Tet1, 2, 3, Sry, Uty—8 alleles) is a high efficient gene-targeting technology from CRISPR/Cas system [4].

Cas9 and the suitably designed sgRNA in transient plasmids can be easily introduced into the target cells and can control several human diseases like diabetes, cancer, schizophrenia, heart disease, and autism. *Streptococcus pyogenes* is one of the pathogenic bacterial species that produce pus in the wound region and show DNA editing system. Some of the programmable molecules acting as site-specific nucleases are Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/cas-based RNA-guided DNA endonucleases that act as therapeutic potential agents [5]. CRISPR studies provide highly advantage research in several microbes rely on diverse defense mechanisms like epidemiological studies, genome typing microbial studies, building specific immunity against undesirable genetic elements, diseased genome and proteome control, host-virus ecological surveys, enhancing viral resistance in domesticated microbes, etc. C-C chemokine receptor type 5 (CCR5) genes in presence of CRISPR/cas9 systems have substantial off-target cleavage resulted in insertions, deletions and point mutations [6, 7].

MATERIAL AND METHODS

Device Specification: Inspiron 3464 AIO; i5, 4GB RAM, x64-based processor.

The analysis for CRISPR and cas9 relationships for understanding mechanisms for gene editing was presented in the work. The analysis is conducted using string database as preliminary level and phylogenetic studies for better species relationships.

The orthology of CRISPR has been analyzed using KEGG database. The number of hits and the genes relevant to orthology has been studied in the present method. The genes that are Ortholous have been submitted to String database to understand the gene interactions of gene editing. The proteins that are relevant to cas9 in *Streptococcus pyogenes* has been searched from string database. The protein structure has been retrieved from PDB based on string database search.

The protein sequence is submitted to BLAST and report was analyzed with refseq_protein as database name with NCBI Protein Reference Sequences as protein description and BLASTP 2.8.1 program. The selected species protein sequences are submitted to the MEGA for the construction of Phylogeny. All the four sequences are also checked with string database and the related protein interaction matches has been analyzed.

RESULTS AND DISCUSSION

The Orthology for CRISPR as per KEGG has provided 55 hits with cas3, csm1, cas10, cas4, cmr4, csm3, cmr3, csn1, cas9, casB, cse2, csa2, cst2, cas7, cmr2, cas10, csa1, csa4, cas8a2, csa5, cst1, cas8a, cas5a_b_c, cas5t, cas6, csh1, csh2, cas5h, csd1, cas8c, csd2, cas5d, csc1, csc2, csc3, casA, cse1, casC, cse4, casD, cse5, casE, cse3, csy1, csy2, csy3, csy4, csb1, csb2, csb3, csx10, csx14, csx17, csn2, csm2, csm4, csm5, cmr5, cmr6, csx1, csx3, csx16, csaX. The word CRISR has not shown any hits in pathway or network. The genes/ proteins shown 20 matches with Humans in String database (Figure 1). STRING found no proteins by this name in Homo sapiens with cas9. Embryonal Fyn-associated substrate (EFS), Casein alpha s1 (CSN1S1), immunoglobulin J polypeptide(IGJ), CSE1 chromosome segregation 1-like (yeast) (CSE1L), transmembrane protein 63C(TMEM63C), Cystatin SN(CST1), Cystatin SA(CST2), Cystatin SA(CSH1), NK2 homeobox 5(NKX2–5), Casein beta(CSN2), NK2 homeobox 3(NKX2–3), Cas scaffolding protein family member 4 (CASS4), Desmin (DES), Chorionic somatomammotropin hormone 2(CSH2), IK cytokine (IK), and Transforming growth factor (TGFBI). Cas9 has better interaction with proteins like DNA polymerase I(polA), NAD-dependent DNA ligase LigA (ligA), Translaldolase (tal), Thymidine kinase (tdk), Streptococcal exotoxin I(speI), Phage associated protein (SPy_0679) and Hypothetical proteins like SPy_1047, SPy_1048, csn2 and SPy_2034 (Figures 2 and 3).

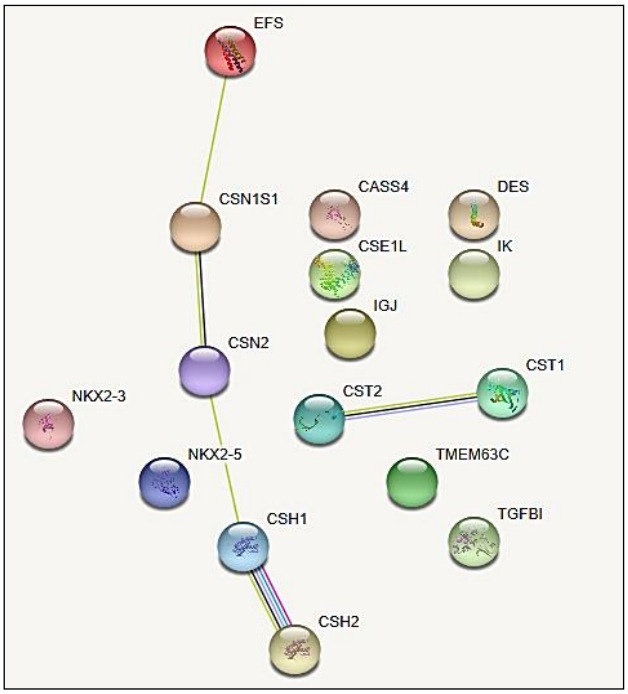

Fig. 1: *Protein Network Approach-based on Orthology from KEGG using string Database for CRISPR.*

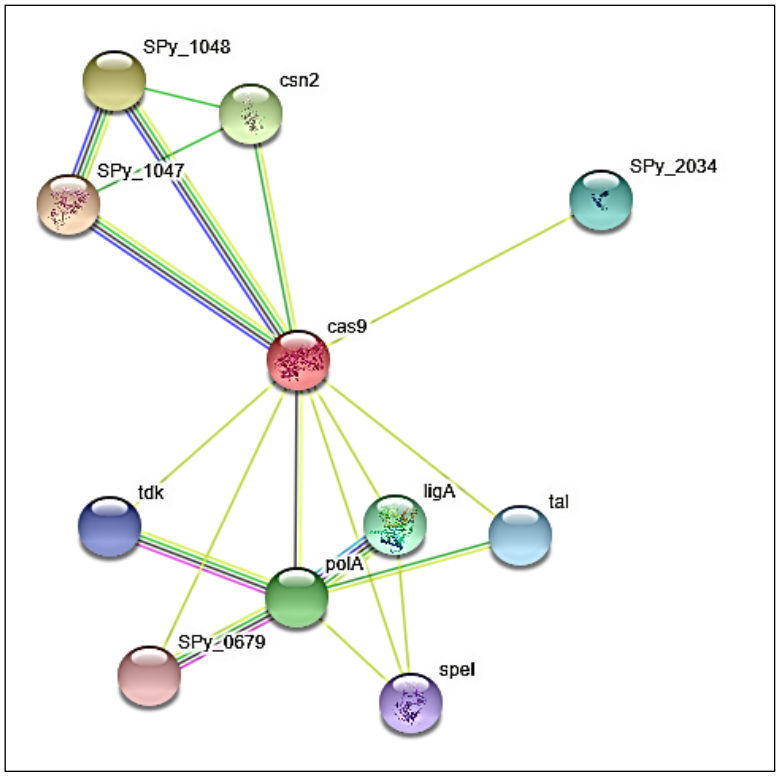

Fig. 2: *Protein Interaction with 10 Molecular Interaction with cas9 using String Database.*

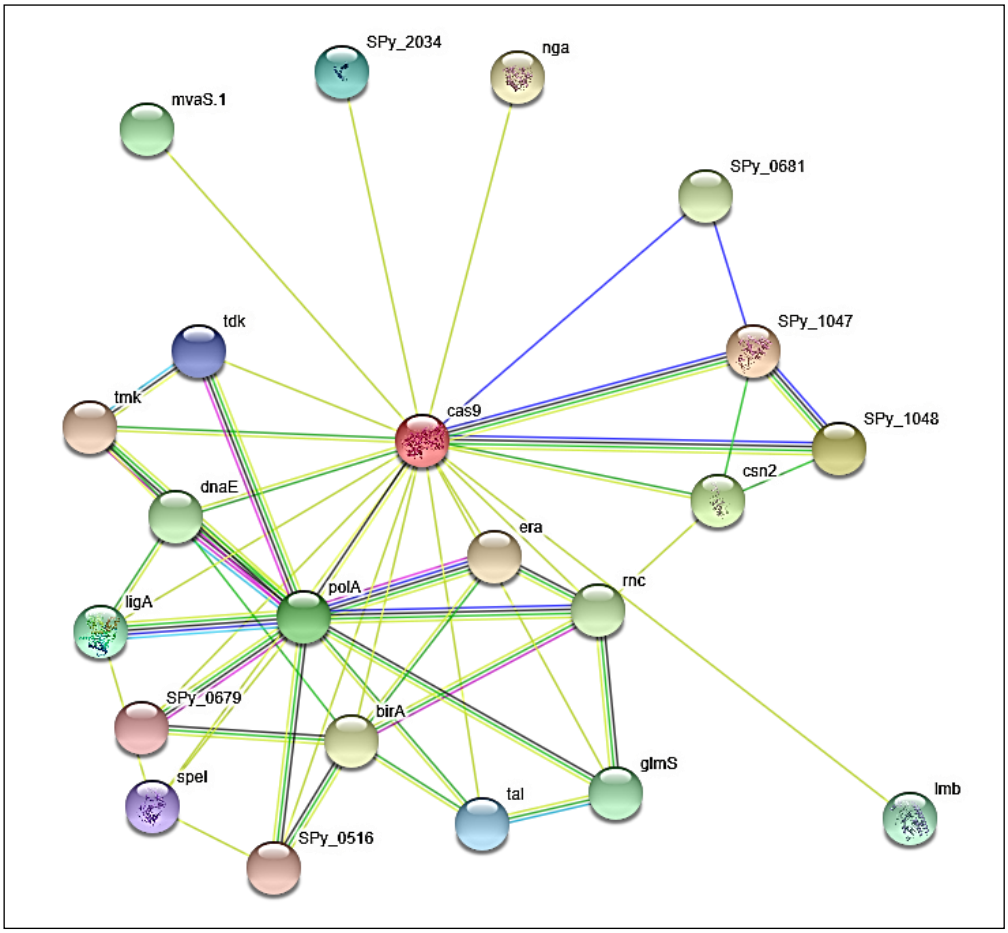

Fig. 3: *Protein Interaction at 50 Molecular Interactions with cas9 using String Database.*

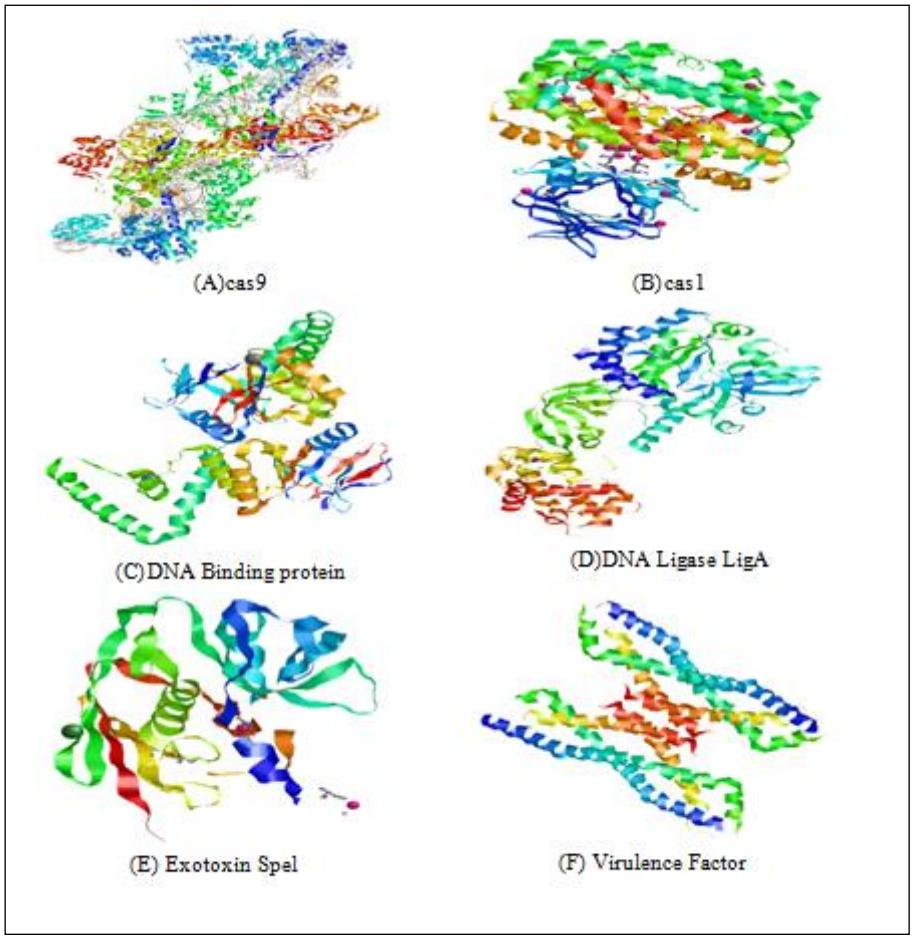

Fig. 4: *(A–F) Protein Molecules showing Interaction in CRISPR Mechanism.*

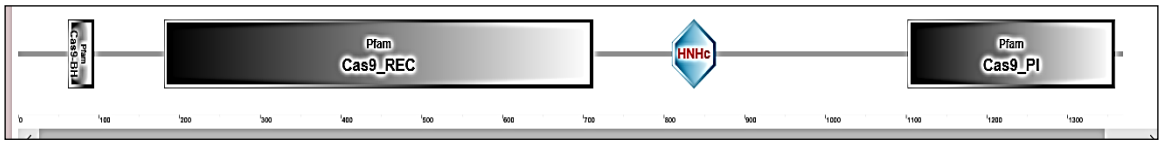

Fig. 5: *Domains within Streptococcus pyogenes serotype M1 Protein CAS9_STRP1 (Q99ZW2).*

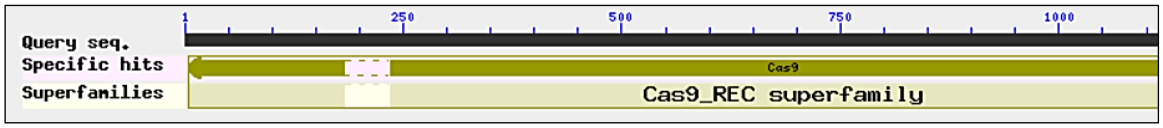

Fig. 6: *Cas9 Superfamily in BLASTp Hits from PDB id 4008.*

The protein structures for the available PDB structures showing interaction in CRISPR mechanism has been viewed using Rasmol is presented in Figure 4. The crystal structure of *Streptococcus pyogenes* Cas9 Figure 4(A) in complex with sgRNA and its target DNA has visualized with bilobed architecture composed of target recognition and nuclease lobes. Figure 5 has shown domains within *Streptococcus pyogenes* serotype M1 protein of cas9.

The FASTA sequence of PDB id 4008 has been retrieved and has conducted further studies. The protein sequence in BLASTp has shown the superfamily as cas9 (Figure 6).

Based on the BLASTp report, the superfamily obtained has been analysed and the 10 best hit sequences has been retrieved with Model organisms. The phylogenetic tree obtained in the MEGA using UPGMA has been shown in Figure 7.

Figure 7 has shown the relationship of *S. pyogenes* with different bacterial species. The species has shown relevance with *Microbacterium arborescens* and *Klebsiella pneumonia*.

The final interaction process of CRISPR/cas9 in DNA editing system has been proposed in Figure 8. In the presence of DNA polymerase I(polA), NAD-dependent DNA ligase LigA (ligA), Translaldolase (tal), Thymidine kinase (tdk), Streptococcal exotoxin I(speI), Phage associated protein (SPy_0679) and Hypothetical proteins like SPy_1047, SPy_1048, csn2 and SPy_2034, the DNA editing will be processed in the Streptococcus pyogenes as per system environmental conditions.

The expression of sgRNA in presence of U6 promoter/SpCas9 under the EFS promoter results in the cleavage of the specific mtDNA loci [8]. Milk protein coding genes, such as CSN1S1, CSN1S2, CSN2, CSN3 and LALBA has shown decrease in expression of β-lactoglobulin (BLG) in BLG knock-out goat mammary glands using CRISPR/Cas9 system [9].

A family of DNA direct repeats (CRISPR) of 21–37 bp is found in many prokaryotic genomes. Four CRISPR-associated (Cas) protein families are strictly associated with Cas1 to Cas4 and are associated as clusters. CRISPR systems belong to different sets of genes, repeat patterns, species range, mobile genetic elements, etc. may show prokaryotic evolution [10]. *in silico* methods may provide better explanation in the interaction mechanisms of biological elements [11–13].

CONCLUSIONS

The interaction of proteins/ genes in the *Streptococcus pyogenes* with DNA editing system with CRISPR/cas9 has been studied in the present work. Further *in vitro* and *in vivo* research has to be conducted for better understanding to propose the mechanism.

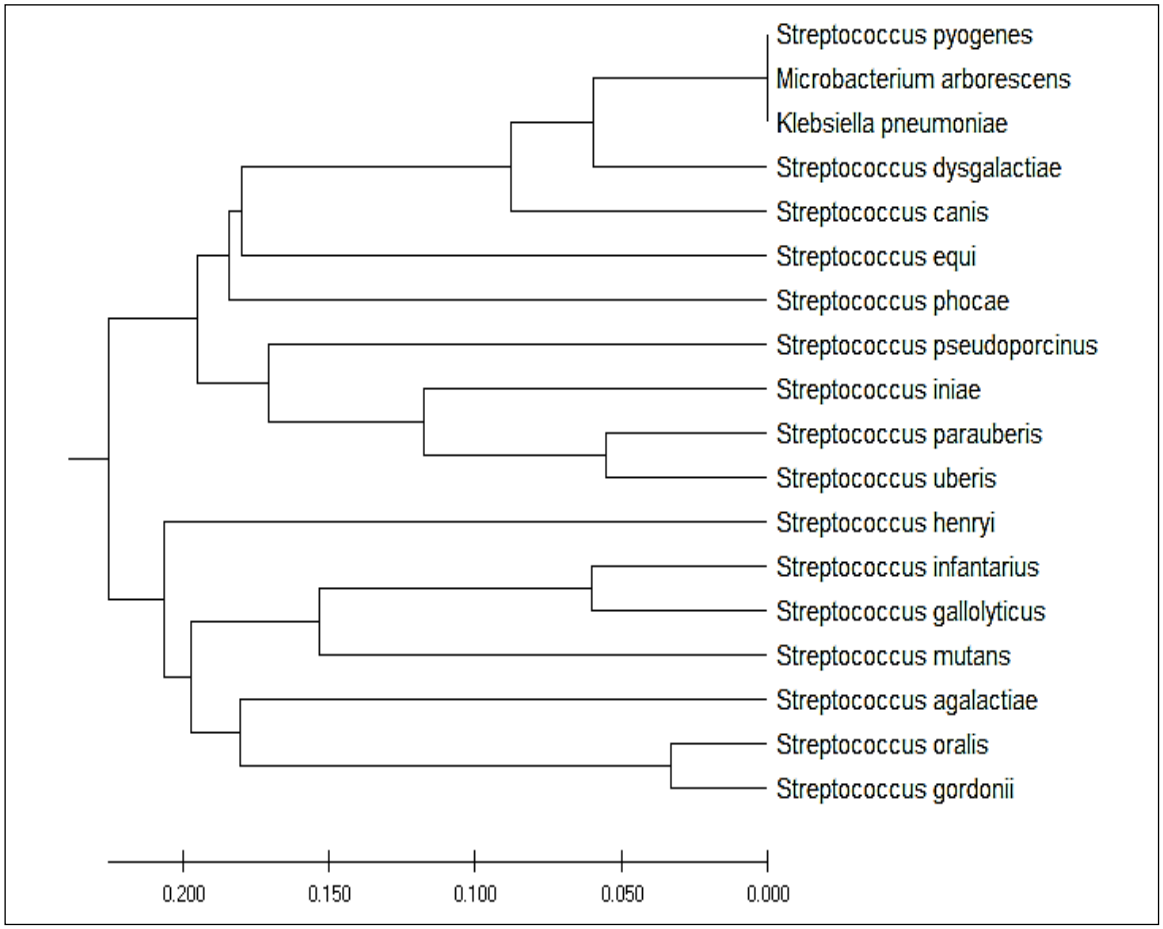

Fig. 7: *Phylogenetic Studies for cas9 in Different Species.*

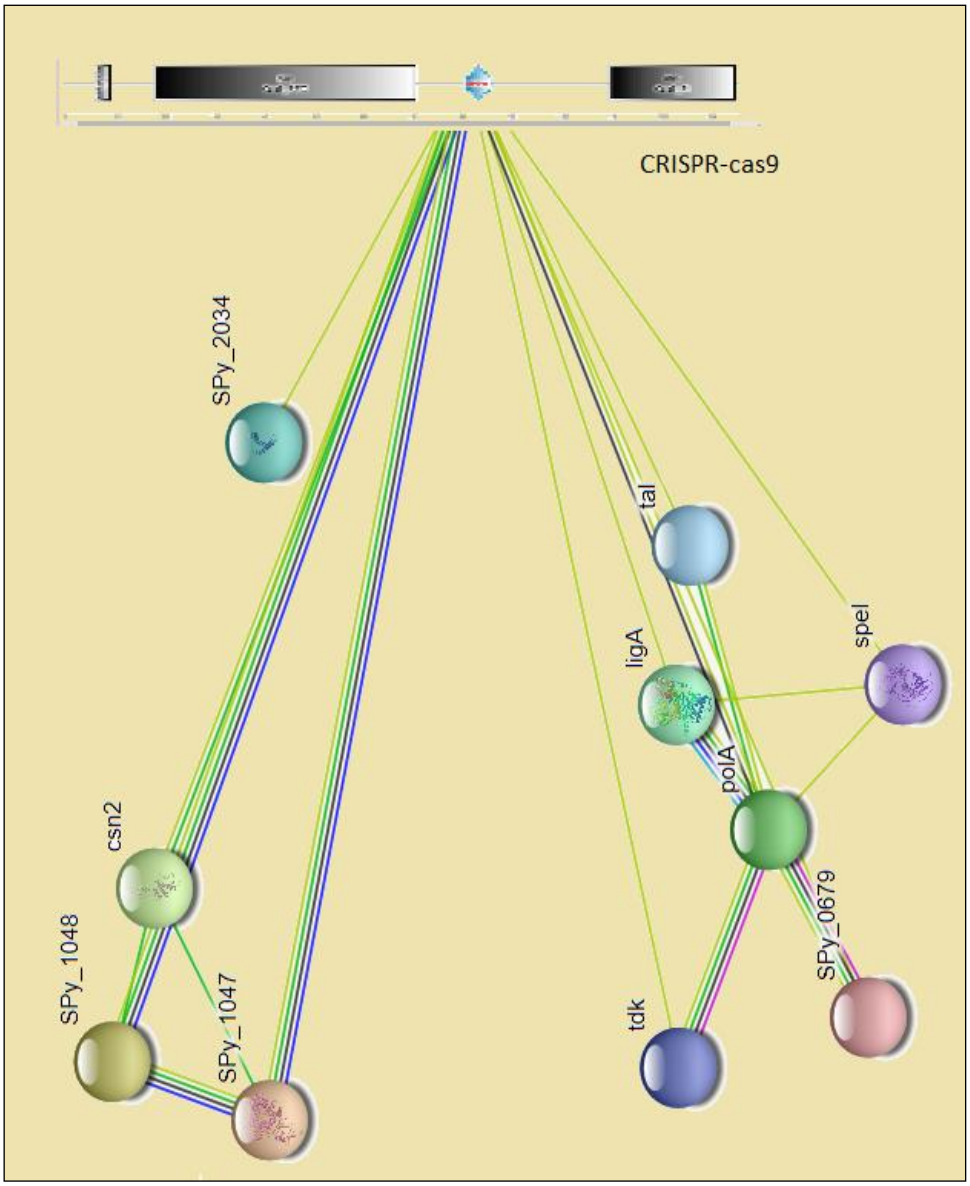

Fig. 8: *Proposed Interaction of CRISPR/cas9 in DNA Editing System.*

CONFLICT OF INTEREST

No conflict of interest.

ACKNOWLEDGEMENT

The author acknowledged and thanking management and staff of Atal Bihari Vajpayee University (formerly Bilaspur University) for support in bringing out the research work.

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Cite this Article
D.S.V.G.K. Kaladha. Studies of CRISPR/CAS 9 Gene Interactions in *Streptococcus pyogenes*. *Research & Reviews: Journal of Computational Biology*. 2018; 7(3): 32–38p.

