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Identification and Characterization of Essential Elements Involved in CRISPR Expression and Maturation in Pathogenic *Leptospirainterrogans*

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Abstract—*Leptospira interrogans, the causative agent of Leptospirosis in humans and animals harbours a CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR associated proteins) system that acts as their adaptive defense strategy to evade foreign genetic material in three stages viz. adaptation, expression and interference.L. interrogans serovar Copenhageni strain Fiocruz L1–130 carries a functional CRISPR-Cas system that belongs to subtype I-B. CRISPR array of this system is flanked by two independent operons of cas genes cluster(cas4-cas1-cas2and cas6-cas3-cas8-cas7-cas5). Orientation of CRISPR array along with its leader sequence was predicted by bioinformatics analysis and validated by RT-PCR.Among these set of Cas proteins, Cas6 of subtype I-B CRISPR-Cas system is known to play a role in the CRISPRRNA maturation, where it processes precursor CRISPR RNA (pre-crRNA) into maturecrRNAs. To characterize Cas6 protein of L. interrogans serovar Copenhageni, cloning and expression of cas6gene (LIC10939) was performed in Escherichia coli using pET expression vector. Recombinant Cas6 protein was purified using nickel-affinity column chromatography and polyclonal antibodies were generated against it in mice. Biochemical studies of recombinant Cas6 revealed that it is a ribonuclease that can cleave structured RNA. Chelation of metal-ions shows Cas6 dependence on divalent-metal (Mg^{2+}) for its RNase activity. Interestingly, Cas6 shows no nuclease activity on DNA substrate but it displays binding propertytoDNA nonspecifically. Additionally, to characterize Cas6 RNase activity on specific RNA substrate, CRISPR array was cloned in pTZ57R/T vector for in vitro synthesis of crRNA. Further study on the mechanism by which Cas6 is involved in the CRISPR maturationin Leptospira may help the biotechnologists in development of novel tools for gene editing inLeptospira and other microbes.*

Keywords*: Leptospira, CRISPR-Cas, Cas6, Ribonuclease, CRISPR RNA.*