INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Name of Thesis Supervisor(s)	:	**Prof. Manish Kumar**
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SHORT ABSTRACT

The pathophysiology of Leptospirosis, caused by pathogenic *Leptospira* spp., is unknown mainly due to the lack of efficient genetic manipulation tools. Thus, harnessing the endogenous CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR associated proteins) system of *Leptospira* is an attractive strategy to study its pathogenesis; however, it relies on the understanding of the CRISPR-Cas immunity process. This study characterizes the CRISPR arrays and CRISPR-associated proteins (LinCas6, LinCas5, and LinCas3) involved in the expression and interference of RNA-mediated immunity. In *L. interrogans*, we account for the transcriptionally active CRISPR arrays in the direction of *cas*-operons. The recombinant LinCas6 (rLinCas6) overexpressed and purified in this study can process the precursor-CRISPR RNA (pre-crRNA) to generate mature crRNA and remains bound with it. The rLinCas6 follows single turnover kinetics where substituting one of the predicted active site residues (His38) reduced cleavage activity on its cognate repeat RNA. Biochemical analysis of the overexpressed and purified rLinCas5 suggested that it is catalytically inactive on nucleic acids. However, rLinCas5 binds to the rLinCas6-crRNA complex essential for stabilizing the mature crRNA during interference. Similarly, the overexpressed and purified rLinCas3 nuclease activity demonstrated that it is a metal-dependent nuclease. This study features insight into CRISPR transcription, crRNA biogenesis, and the onset of the effector complex formation in *Leptospira*, which is essential for RNA-mediated interference of invading nucleic acids. In addition, this study proposes the physiological requirements of *Leptospira* CRISPR-Cas I-B during interference.