"Functional and Evolutionary Insights into Dual CRISPR-Cas Systems in the Halophilic Archaeon *Haloferax lucentense*"

By Doron Naki under the supervision of Prof. Uri Gophna

Microorganisms interact with various mobile genetic elements (MGEs) in complex ways that encompass both cooperation and competition. Prokaryotes have evolved adaptive immune mechanisms to counter MGEs, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) systems. In this study, we explore the intriguing CRISPR-Cas system dynamics within the halophilic archaeon, *Haloferax lucentense*, which harbors two distinct type I-B CRISPR-Cas systems: a complete type I-B system on a plasmid and a second system encoded on a provirus that lacks the adaptation module necessary for new spacer acquisition. Our main objective is to understand the interaction between these two systems and their functional integration during viral infection.

Through spacer sequence analysis and comparative genomics, we identified intricate interactions between these systems. Upon infecting *H. lucentense* with HFPV-1 (*Haloferax volcanii* pleomorphic virus 1), we observed new spacer acquisition across all CRISPR arrays, indicating that the plasmid-based adaptation module acts as a backup to support the proviral CRISPR system. This research provides insights into the diversity of CRISPR-Cas systems, their roles in prokaryotic immunity, and the broader ecological and evolutionary dynamics between hosts and parasites.

Main points:

1. **Characterization and Functionality of Dual CRISPR-Cas Systems in *H. lucentense***
   * analysis of interference and the role of plasmid and provirus CRISPR-Cas systems.
2. **Spacer Acquisition and Anti-Viral Defense**
   * analysis of spacer acquisition after viral infection (HFPV-1).
3. **Eco-Evolutionary Perspectives and Broader Implications**
   * Comparative analysis with other archaea, highlighting host-parasite co-evolution dynamics and CRISPR-Cas diversity.