INVESTIGATION OF THE ACTIVATION MECHANISM OF SPLITPAGO PROTEINS AND ASSOCIATED HEPN EFFECTORS IN E. COLI

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As genome editing technologies progress, the scientific community is committed to enhancing the tools that facilitate this development. Presently, CRISPR-Cas systems lead the way in genome editing technologies, while limited research has been dedicated to their counterparts – pAgo systems. Nonetheless, recent discoveries regarding Argonaute protein research suggest that the HEPN domain is vital for the function of certain Argonaute systems [1], but their activation mechanisms are still unknown.

This research explores the potential interaction between prokaryotic Argonaute (pAgo) system and a plasmid with a CloDF13 origin of replication *(ori)* sequence. The pAgo system examined in this research includes a toxic effector protein, which contains a HEPN domain. This effector protein exhibits toxicity in the absence of its associated pAgo proteins. In some cases, pAgo systems can be activated by plasmids carrying a specific CloDF13 *ori* [2]. Through the investigation of an Argonaute–HEPN system with CloDF13 *ori* acting as a trigger, we aim to explore the activation mechanism of this system. This research not only lays the groundwork for future studies but also possesses significant potential for programmable DNA targeting in the field of biotechnology.

Investigating bacterial defense activation mechanisms provides critical insight of the intricate ways in which bacteria safeguard themselves against foreign DNA while also creating opportunities for advancements in biotechnology and genome editing tools.

[1] M. C. Pillon, K. H. Goslen, J. Gordon, M. L. Wells, J. G. Williams, and R. E. Stanley, "It takes two (Las1 HEPN endoribonuclease domains) to cut RNA correctly," Journal of Biological Chemistry, vol. 295, no. 18, pp. 5857–5870, Mar. 2020, doi: 10.1074/jbc.ra119.011193.

[2] M. Zaremba et al., "Short prokaryotic Argonautes provide defence against incoming mobile genetic elements through NAD+ depletion," Nature Microbiology, vol. 7, no. 11, pp. 1857–1869, Oct. 2022, doi: 10.1038/s41564-022-01239-0.