High-throughput tagging of endogenous loci for rapid characterization of protein function

Joonwon Kim

University of California, San Diego
La Jolla, California 92093 jok038@health.ucsd.edu

Understanding the dynamic behavior of proteins and their interacting partners is essential for deciphering the inner workings of cells. Protein tagging serves as a powerful tool to track protein behaviors and identify their interaction partners. However, current tagging methods are often labor-intensive and limited to tagging one gene at a time within mammalian cells. To address these limitations, we have developed High-throughput Insertion of Tags Across the Genome (HITAG), a Cas9-based targeted insertion strategy for the rapid construction of cell libraries with different proteins of interest at the C-terminus. HITAG exhibits high tagging specificity, with the majority of tagging events being indel-free. To demonstrate the utility of HITAG, we generated a pool of 244 mCherry-tagged cell lines for stress granule studies and isolated 167 kinds of clonal cell lines for further analysis. This approach allows us to investigate proteins and elucidate the features that drive a subset of proteins to strongly accumulate within these transient RNA-protein granules. We will share the general principles of HITAG and discuss how to apply HITAG to accelerate the rate of protein studies.