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From protein-centered to gene-centered approaches to investigate DNA-protein interactions in grapevine.

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Abstract

DNA-binding proteins play a pivotal role in critical cellular processes such as DNA replication, transcription, recombination, repair, and other essential activities. Consequently, investigating the interactions between DNA and proteins is of paramount importance to gain insights into these fundamental cellular mechanisms. Several methodologies have been devised to uncover DNA-protein interactions, which can be broadly categorized into two approaches. The “protein-centered” approach focuses on identifying the DNA sequences bound by a specific transcription factor or a set of TFs. Techniques falling within this category include chromatin immunoprecipitation, and protein-binding microarrays. The "gene-centered" approach entails using one or more DNA sequences as bait to explore the TFs that bind to these specific DNA elements. Methods belonging to this approach encompass yeast one-hybrid (Y1H), PICh, and Reverse ChIP. Both methodologies offer distinct advantages and face limitations, largely stemming from challenges related to complexity, efficiency, and specificity. With the emergence of next-generation sequencing (NGS) protocols and the CRISPR/Cas system, new avenues for investigating trans-cis interactions in organisms have opened. In our research focusing on grapevines, we discuss advancements in both protein- and gene-centered approaches. Firstly, we present the implementation of a DNA Affinity Purification (DAP-seq) protocol in grapevines to explore the cistrome associated with various TFs from the WRKY and MYB families. Secondly, we address the challenge of developing an innovative gene-centered approach utilizing a CRISPR/Cas system for in-situ purification of regulatory elements. This approach aims not only to identify proteins associated with specific genomic regions but also to elucidate long-range DNA interactions.

Keywords: DAP-seq, Grapevine, Gene Regulation, CRISPR-Cas9, Protoplasts.