

DEVELOPING A PROTOCOL TO ISOLATE TRANSCRIPTION FACTORS BOUND TO A SPECIFIC DNA LOCUS FROM THE EXTRACTED CHROMATIN OF GRAPEVINE PROTOPLASTS USING CRISPR-DCAS9 SYSTEM

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Cis-acting regulatory elements (CREs) are DNA sequences that can be bound by transcription factors (TF) to finely regulate the transcription of protein-coding and non-coding RNAs in a condition-dependent and tissue-specific way. It is nowadays possible to search for DNA motives and sequences that a given transcription factor is binding or at least can, but it is still hard to have a glance at all the transcription factors that are contemporaneously located at the same locus. Inspired by a technique that aims at solving this problem in mammal cells using the well-known CRISPR-Cas system, we are trying to develop a protocol to study such gene regulation in plants of the *Vitis* species. Making use of the highly sequence-specific binding capacity of a catalytically inactive Cas9 protein (dCas9), our idea is to set up a system to target a desired sequence and precipitate all the crosslinked proteins and distantly interacting chromatin at this locus and analyze them. The simplicity of protoplasts, especially for transfection, makes them widely used for biotechnology applications and proofs of concepts. This will be convenient to us if we want to transiently express the dCas9 and guide-RNA instead of transforming them with a preassembled ribonucleoprotein. Here we present the different issues we went through while establishing our protocol, starting with the current rarity of such approaches in literature, and the solutions we came up with.