Preliminary steps of a protocol to isolate transcription factors bound to a specific DNA locus in grapevine using CRISPR-dCas9 system.

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Abstract (250 words)

Cis-acting regulatory elements are DNA sequences that can be bound by transcription factors to regulate the expression of genes 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 an existing technique that uses the CRISPR-Cas system in mammal cells, we are trying to develop a protocol to study such regulation in *Vitis vinifera*. Using 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. After conducting preliminary assays on protoplast system, we got introduced to the CRISPR-FISH technique, that uses dCas9 and a fluorescent guide to label telomeres on nuclei directly isolated from a small quantity of fixed leaves with a very simple procedure. We used part of such technique to easily obtain the chromatin that was needed for our trials and eventually performed the pull-down of the targeted DNA sequences directly on these nuclei after transforming them with the dCas9 complex. Sequencing the obtained fragments allows to verify the specificity of the tool. Far from having eliminated the idea of using protoplasts as model system, we proceed developing both approaches in parallel.

Keywords: Molecular Biology, Grapevine, Gene Regulation, CRISPR-Cas9, Protoplasts.