Gene functional characterization assisted by genome-wide TF-binding site interrogation: Grapevine as a case of study

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Abstract

The study of transcriptional regulation through protein-DNA binding exploring methods can provide an outline of the roles a transcription factor (TF) may carry out based on what coding genes are bound in their promoter regions. In non-model plant species, TFs can be now examined in their genome-wide DNA-binding profiles (cistromes) by the in vitro technique DAP-seq, which involves expressing a tagged TF and then immobilizing it for subsequent exposure to genomic DNA fragments. The technique offers important advantages with respect to ChIP-seq (no need to develop a TF-specific antibody or generate transgenic organisms expressing a tagged TF). This is of particular benefit when working on slow-growing non-model organisms, where genetic transformation is time-consuming. Our group has implemented the DAP-seq method in grapevine to identify all the sites in the genome where transcription factors bind. Depending on each case we have been able to obtain thousands of binding events assigned to genes, with important TF family-specific differences of peak location distributions. The possibility of performing stable or transient over-expression experiments constitutes a second step in gene target discovery, as shown in the validation of MYB15/MYB14, HY5, MYBA7/A1 and MYBPA1 high confidence targets, or in the characterization of the main regulator of the onset of fruit ripening CARPO. In addition, to TF function discovery, DAP-seq also helped us to hypothesize and explore TF-TF interactions and to identify novel pathway genes that can later be characterized. To visualize the cistromes of different TFs we have created 'DAPBrowse', currently displayed in the Vitis Visualization platform, a publicly available resource with a range of analytical and visualization tools for grapevine. For the initial inspection of TF targets we demonstrate how bound genes can be overlapped with co-expression networks to look for potential targets. Finally, applying DAP-seq in grape calls for species-specific experimental considerations which are being gathered in a set of guidelines currently under preparation in the frame of the Integrape COST Action. The main aim of the guidelines is to provide a standardised framework for DAP-seq experiments in grapevine as well as to help the community in the preparation and analysis of experiments.

Keywords: DNA affinity purification sequencing, transcription factor, binding landscape, gene characterization