

127. SND1 Transcription Factor–Directed Quantitative Functional Hierarchical Genetic Regulatory Network in Wood Formation in *Populus trichocarpa*

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Wood is an essential renewable raw material for industrial products and energy. However, knowledge of the genetic regulation of wood formation is limited. We developed a genome-wide high-throughput system for the discovery and validation of specific transcription factor (TF)– directed hierarchical gene regulatory networks (hGRNs) in wood formation. This system depends on a new robust procedure for isolation and transfection of *Populus trichocarpa* stem differentiating xylem protoplasts. We overexpressed Secondary Wall-Associated NAC Domain 1s (Ptr-SND1-B1), a TF gene affecting wood formation, in these protoplasts and identified differentially expressed genes by RNA sequencing. Direct Ptr-SND1-B1–DNA interactions were then inferred by integration of timecourse RNA sequencing data and top-down Graphical Gaussian Modeling–based algorithms. These Ptr-SND1-B1-DNA interactions were verified to function in differentiating xylem by anti-PtrSND1-B1 antibody-based chromatin immunoprecipitation (97% accuracy) and in stable transgenic *P. trichocarpa* (90% accuracy). In this way, we established a Ptr-SND1-B1–directed quantitative hGRN involving 76 direct targets, including eight TF and 61 enzyme-coding genes previously unidentified as targets. The network can be extended to the third layer from the second-layer TFs by computation or by overexpression of a second-layer TF to identify a new group of direct targets (third layer). This approach would allow the sequential establishment, one two-layered hGRN at a time, of all layers involved in a more comprehensive hGRN. Our approach may be particularly useful to study hGRNs in complex processes in plant species resistant to stable genetic transformation and where mutants are unavailable.

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