A new system for fast and quantitative analysis of heterologous gene expression in plants

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Résumé en anglais	Large-scale analysis of transcription factor–cis-acting element interactions in plants, or the dissection of complex transcriptional regulatory mechanisms, requires rapid, robust and reliable systems for the quantification of gene expression.Here, we describe a new system for transient expression analysis of transcription factors, which takes advantage of the fast and easy production and transfection of Physcomitrella patens protoplasts, coupled to flow cytometry quantification of a fluorescent protein (green fluorescent protein). Two small-sized and high-copy Gateway® vectors were specifically designed, although standard binary vectors can also be employed. As a proof of concept, the regulation of BANYULS (BAN), a key structural gene involved in proanthocyanidin biosynthesis in Arabidopsis thaliana seeds, was used. In P. patens, BAN expression is activated by a complex composed of three proteins (TT2/AtMYB123, TT8/bHLH042 and TTG1), and is inhibited by MYBL2, a transcriptional repressor, as in Arabidopsis. Using this approach, two new regulatory sequences that are necessary and sufficient for specific BAN expression in proanthocyanidin-accumulating cells were identified. This one hybrid-like plant system was successfully employed to quantitatively assess the transcriptional activity of four regulatory proteins, and to identify their target recognition sites on the BAN promoter.
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