

## **Plant ACE hormometer: a multi-hormone reporter to detect and quantify auxin, cytokinin and ethylene**

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Phytohormones are essential regulatory molecules that guide plant development. As sessile organisms, plants integrate environmental changes and internal signals by changing the balance in hormone concentrations, thereby adapting plant growth, development, or defense responses in tune with the environment. Despite the importance of hormone signaling in plant development, it remains technically challenging to detect and quantify multiple hormones simultaneously, which negatively impacts our understanding of the hormone crosstalk that occurs in plants in response to different biotic and abiotic stresses. To overcome this constraint, our laboratory has leveraged synthetic biology tools to generate a library of GoldenBraid-domesticated DNA parts. Our collection includes over a hundred entry clones harboring minimal promoters and terminators, hormone-specific distal promoters, sequence-divergent fluorescent, luminescent, and histochemical marker proteins, and different subcellular localization signals. These DNA elements can be rapidly assembled together in variable combinations using GoldenBraid to generate multi-hormone single-locus reporters. By combining three variants of three fluorescent proteins (mTagBFP2, YPet, and mCherry) targeted to three distinct subcellular compartments, theoretically, a construct with up to nine compatible biosensors can be generated to detect nine major classes of plant hormones in parallel. Thus far, we have built several versions of a triple reporter construct to monitor auxin, ethylene, and cytokinin by utilizing novel versions of auxin (10xDR5v2), ethylene (EBSnew), and cytokinin-inducible (TCSn1) distal promoters. The reporters were transformed into Arabidopsis and tomato and homozygous transgenic lines were characterized. We will discuss the library of resources we have built and the constructs that have been functionally validated. The feasibility of our approach will be demonstrated through the characterization of Arabidopsis and tomato triple reporter lines.