A single genome gives rise to different cell types and organs in response to precise temporal and spatial regulation of gene expression, driven by developmental and environmental cues. These expression patterns are orchestrated by cis-regulatory elements, distal enhancers, and gene-proximal promoters. Active enhancer elements can be identified by Self-Transcribing Active Regulatory Region Sequencing (STARR-seq), a massively parallel enhancer reporter assay initially developed in Drosophila (Arnold et al., 2014) and previously applied in rice and maize protoplasts (Sun et al., 2019; Ricci et al., 2019).

In a new study, Jores et al. bypass these limitations by establishing an optimized STARR-seq assay in tobacco (Nicotiana benthamiana). Transient expression of the STARR-seq libraries is achieved by efficient Agrobacterium-mediated transformation through syringe-infiltration of intact tobacco leaves. To validate the specificity and sensitivity of the STARR-seq assay in tobacco, the authors designed a reporter construct containing a barcoded GFP reporter gene under the control of a Cauliflower mosaic virus 35S minimal promoter and a 35S core enhancer (Figure). Candidate enhancer elements can be inserted at different positions within the construct. Each candidate enhancer is uniquely linked to a barcoded GFP reporter, providing a specific and quantitative readout for the activity of the element revealed by RNA-seq.

The authors first tested the dynamic range of the tobacco STARR-seq assay by systematically analyzing the positional effect of the 35S enhancer on reporter gene transcription. As expected, the 35S enhancer is orientation-insensitive and exerts distance-dependent transcripional enhancement when located either upstream or downstream of the reporter gene. Addition of an extra 35S enhancer copy appreciably increased the transcriptional output, with a stronger effect for downstream and distal upstream enhancers. By contrast, the 35S enhancer displayed minimal-to-null activity when located in transcribed regions such as the 3′-UTR, in contrast with previous reports in rice and maize protoplasts. To address whether this finding is specific to the tobacco system, the authors transformed maize protoplasts, finding again that placement within the transcribed region resulted in little signal.

The authors further tested the suitability of the assay for the functional dissection of such cis-regulatory elements. To answer this question, all possible mutations within the 35S promoter and enhancer were generated, and the effect of individual mutations on the reporter gene transcription scored in both contexts. Mutations in the 35S enhancer showed a weak-to-no effect on transcription, with the exception of the TATA box. By contrast, saturation mutagenesis of the 35S enhancer identified multiple nucleotides critical for the activity of the cis-regulatory element, thus revealing functional sub-motifs.

The tobacco STARR-seq assay relies on reporter constructs driven by the 35S minimal promoter (green). Candidate enhancers (blue) are inserted at different positions within the construct. Barcodes (shades of purple) are inserted into the GFP open reading frame and used as a quantitative readout of the transcriptional enhancer activity after RNA-seq on Agrobacterium-transformed tobacco leaves. BlpR, phosphinothricin resistance gene; pA site, poly-adenylation site. (Adapted from Jores et al [2020], Figure 1.)
within the enhancer. Most of the functional sub-motifs overlapped with transcription factor binding sites. Lastly, systematic combinations of the functional sub-domains forming synthetic enhancers demonstrated modular and non-additive enhancer activity, supporting the potential of the tobacco STARR-seq assay for building synthetic regulatory elements.

The high conservation of transcription factors in the plant lineage paves the way for the tobacco STARR-seq system to be a powerful and versatile approach for in-depth dissection of regulatory sequences in many plant genomes. The continuous development of such methods opens new possibilities in the genetic engineering of regulatory elements and ultimately in the dissection of the gene regulatory networks orchestrating plant development.

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REFERENCES
Arnold, C.D., Gerlach, D., Spies, D., Matts, J.A., Sytnikova, Y.A., Pagani, M., Lau, N.C., and Stark, A. (2014). Quantitative genome-wide enhancer activity maps for five Drosophila species show functional enhancer conservation and turnover during cis-regulatory evolution. Nat. Genet. 46: 685–692.

Ricci, W.A. et al. (2019). Widespread long-range cis-regulatory elements in the maize genome. Nat. Plants 5: 1237–1249.

Sun, J., He, N., Niu, L., Huang, Y., Shen, W., Zhang, Y., Li, L., and Hou, C. (2019). Global Quantitative Mapping of Enhancers in Rice by STARR-seq. Genomics. Proteomics Bioinformatics 17: 140–153.

Jores, T., Tonnies, J., Dorrity, M.W., Cuperus, J.T., Fields, S. and Queitsch, C. (2020). Identification of plant enhancers and their constituent elements by STARR-seq in tobacco leaves. Plant Cell DOI: https://doi.org/10.1105/tpc.20.00155.
Shooting for the STARRs: a modified STARR-seq assay for rapid identification and evaluation of plant regulatory sequences in tobacco leaves

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