

Systems and Synthetic Biology

Siobhan Brady,
sbrady@ucdavis.edu
 University of California, Davis



with support from
 Gloria Coruzzi, gc2@nyu.edu, New York University
 Miriam Gifford, M.L.Gifford@warwick.ac.uk, University of Warwick
 Nicola Patron, Nicola.patron@earlham.ac.uk, Earlham Institute

Recently developed Open Tools and Resources for Arabidopsis Researchers

(i) Belcher, M.S., Vuu, K.M., Zhou, A. *et al.* Design of orthogonal regulatory systems for modulating gene expression in plants. *Nat Chem Biol* 16, 857–865 (2020).

A library of synthetic transcriptional regulators (activators, repressors and promoters) that modulate expression strength in planta.

(ii) Brooks MD, Juang C-L, Katari MS, Alvarez JM, Pasquino H-J, Huang J, Shanks C, Cirrone J, Coruzzi GM. *ConnectTF: A platform to integrate transcription factor–gene interactions and validate regulatory networks.* *Plant Physiology*. 185(1). (2021)

A species-independent, web-based platform that integrates genome-wide studies of TF-target binding, TF-target regulation and other TF-centric omic datasets and uses these to build and refine validated or inferred gene regulatory networks.

(iii) Cai YM, Carrasco Lopez JA, Patron NJ. *Phytobricks: Manual and Automated Assembly of Constructs for Engineering Plants.* *Methods in Molecular Biology*. 2205:179-199.

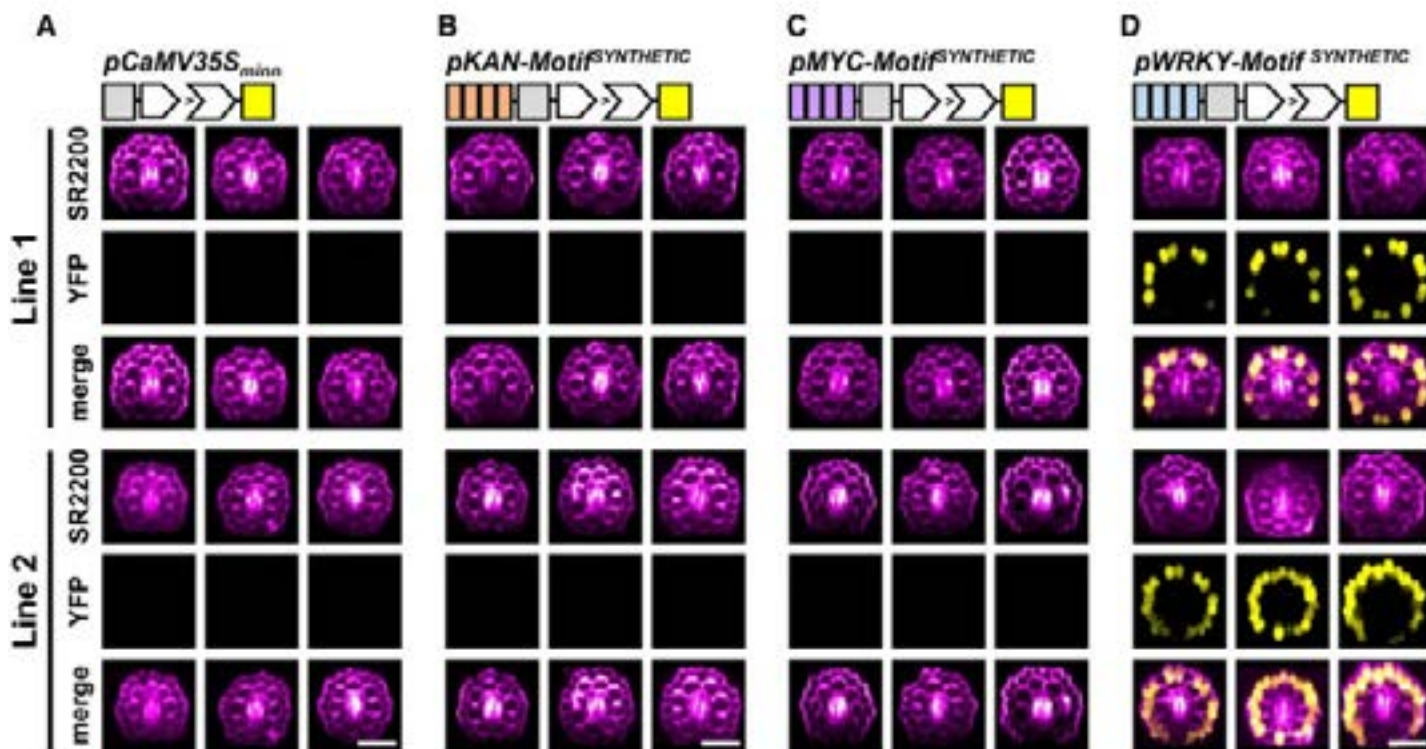
Phytobricks are standardized DNA parts for plants that can be assembled hierarchically into transcriptional units and multigene constructs. This protocol describes Phytobrick design and construction and their assembly in manual and nanoscale automated one-step reactions as well as high-throughput sequence verification of assembled plasmids.

(iv) Lehmann S., Dominguez-Ferreras A., Huang W., Denby K., Ntoukakis V., Schäfer P. Novel markers for high-throughput protoplast-based analyses of phytohormone signaling. *Plos One* 15: e0234154 (2020)

The authors generated and validated 18 promoter::luciferase and suggest an experimental setup for high-throughput analysis. They recommended novel markers for the analysis of auxin, abscisic acid, cytokinin, salicylic acid and jasmonic acid responses.

(v) Cirrone J, Brooks MD, Bonneau R, Coruzzi GM, Shasha DE. *OutPredict: multiple datasets can improve prediction of expression and inference of causality.* *Scientific Reports*. 10(1):6804. (2020)

Prediction of causal gene regulatory relationships based on time series data as well as known network edges and steady-state data. The method across different species has improved predictive accuracy over other state-of-the-art methods.



“Motif-Only” Synthetic Promoters Reveal Regulatory Functions of Promoter Motifs in Gene Expression in Planta.

Synthetic promoters only consisting of a set of four motifs or motif combinations and a CaMV35S minimal promoter (but lacking any native promoter backbone sequence) were analyzed in plant lines stably expressing the Promoter:LhG4 > pOp6:YFP transactivation system. Except for WRKY-KANADI motif combinations, two independent plant lines were analyzed per promoter construct by confocal microscopy of axial root sections.

(A) to (D) Plant lines expressing the CaMV35S minimal promoter (A), 4× KANADI binding motifs (B), or 4× MYC binding motifs (C) did not activate YFP expression, whereas plant lines expressing 4× WRKY resulted in epidermis-specific YFP expression (D).

Recent or Future activities of Subcommittee members

(i) (Plant session at) 4th German Conference on Synthetic Biology, 24 & 25 September 2020, Virtual

(ii) 4th International Conference on Plant Synthetic Biology, Bioengineering, and Biotechnology October 30 - November 1, 2020, Virtual

(iii) Cold Spring Harbor Network Biology Meeting; March 16-19, 2021; Virtual; Organizers included Pascal Falter-Braun, a member of this subcommittee

(iv) Second iPSB: EMBO Workshop on International Plant Systems Biology: April 26-27, 2021; Main Organizers: Katherine Denby and Miriam Gifford

(v) 31st March 2021 Plantae SynBio, Virtual

Selected Publications

(i) Altmann M, Altmann S, Rodriguez PA, Weller B, Wlorduy Vergara L, Palme J, Marín-de la Rosa N, Sauer M, Wenig M, Villaécija-Aguilar JA, Sales J, Lin CW, Pandiarajan R, Young V, Strobel A, Gross L, Carbonnel S, Kugler KG, Garcia-Molina A, Bassel GW, Falter C, Mayer KFX, Gutjahr C, Vlot AC, Grill E, Falter-Braun P. Extensive signal integration by the phytoproteome protein network. *Nature*. 583(7815):271-276.

A systems-level map of the Arabidopsis phytohormone signalling network.

(ii) Alvarez JM, Schinke A-L, Brooks MD, Pasquino A, Leonelli L, Varala K, Safi A, Krouk G, Krapp A, Coruzzi GM. Transient genome-wide interactions of the master transcription factor NLP7 initiate a rapid nitrogen-response cascade. *Nature Communications*. 11(1157). 2020

Capture of stable and transient TF-target interactions of NLP7, a master regulator of the nitrogen signaling pathway, using time-series ChIPseq and DamID-seq. These approaches can be applied to validate dynamic GRN models for any pathway of interest.

(iii) Cai YM, Kallam K, Tidd H, Gendarini G, Salzman A, Patron NJ. Rational design of minimal synthetic promoters for plants. *Nucleic Acids Research*. 48(21):11845-11856. (2020)

Design of a suite of synthetic promoters of different strengths that can regulate the relative expression of output genes in simple genetic devices.

(iv) Rich-Griffin C, Eichmann R, Reitz MU, Hermann S, Woolley-Allen K, Brown PE, Wiwatdirekkul K, Esteban E, Pasha A, Kogel KH, Provart NJ, Ott S, Schäfer P. Regulation of Cell Type-Specific Immunity Networks in Arabidopsis Roots. *Plant Cell*. 32:2742-2762. (2020)

Identification of gene networks activated by two immunity elicitors (flg22 and Pep1) in epidermis cortex and pericycle cells of Arabidopsis. A tool to analyze paired transcription factor binding motifs was also developed.

(v) Smit ME, McGregor SR, Sun H, Gough C, Bågman AM, Soyars CL, Kroon JT, Gaudinier A, Williams CJ, Yang X, Nimchuk ZL, Weijers D, Turner SR, Brady SM, Etchells JP. A PXY-Mediated Transcriptional Network Integrates Signaling Mechanisms to Control Vascular Development in Arabidopsis. *Plant Cell*. 32(2):319-335. (2020)

A PXY-mediated transcriptional network was mapped which revealed a feedforward loop incorporating WOX14, TMO6 and LBD4. These integrate both auxin and PXY signaling to determine the phloem-procambium boundary in vascular tissue.

Planning for Fourth Decadal Roadmap.

- (i) Translation of Arabidopsis research to crop plants – including close relatives like other Brassicas, and other relatives like tomato and more distantly related dicots
- (ii) Model-guided predictable engineering of complex traits.
- (iii) Spatial and temporal dynamics in plant biology.