Quantitative Biology 2023, 11(3): 214–230
https://doi.org/10.15302/J-QB-022-0326

REVIEW

From qualitative to quantitative: the state of the art and challenges for plant synthetic biology

Chenfei Tian[1,2], Jianhua Li[1,*], Yong Wang[1,*]

[1] CAS-Key Laboratory of Synthetic Biology, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China
[2] University of Chinese Academy of Sciences, Beijing 100039, China
* Correspondence: yongwang@cemps.ac.cn; jhli@cemps.ac.cn

Received August 1, 2022; Revised December 24, 2022; Accepted March 1, 2023

***Backgrounds*: As an increasing number of synthetic switches and circuits have been created for plant systems and of synthetic products produced in plant chassis, plant synthetic biology is taking a strong foothold in agriculture and medicine. The ever-exploding data has also promoted the expansion of toolkits in this field. Genetic parts libraries and quantitative characterization approaches have been developed. However, plant synthetic biology is still in its infancy. The considerations for selecting biological parts to design and construct genetic circuits with predictable functions remain desired.**
***Results*: In this article, we review the current biotechnological progresses in field of plant synthetic biology. Assembly standardization and quantitative approaches of genetic parts and genetic circuits are discussed. We also highlight the main challenges in the iterative cycles of design-build-test-learn for introducing novel traits into plants.**
***Conclusion*: Plant synthetic biology promises to provide important solutions to many issues in agricultural production, human health care, and environmental sustainability. However, tremendous challenges exist in this field. For example, the quantitative characterization of genetic parts is limited; the orthogonality and the transfer functions of circuits are unpredictable; and also, the mathematical modeling-assisted circuits design still needs to improve predictability and reliability. These challenges are expected to be resolved in the near future as interests in this field are intensifying.**

Keywords: plant synthetic biology; quantitative characterization; genetic parts; genetic circuits

Author summary: The flourishing plant science promotes the exploding number of data and the expansion of toolkits. Plant synthetic biology is still in its early stages and requires more quantitative and predictable study. Despite the challenges, some pioneering examples have been successfully demonstrated in model plants.

PLANT SYNTHETIC BIOLOGY: THE CURRENT STATUS

As a bottom-up approach, synthetic biology is a multidisciplinary field aiming to rewire a biological process/behavior and design/build man-made life systems for the benefit of human beings. Simple biological parts are assembled together into complex genetic circuits to obtain programmed cells or organisms. Tremendous progress have been made in prokaryotic microbial systems [1,2], the next wave of synthetic biology has now advanced from single-celled organisms to complicated multicellular systems.

Plants are attractive platforms for engineered traits because of their advanced ability to adapt to living environment, variety of growth forms, and their natural ability to produce useful metabolites. Plant synthetic biology includes not only the introduction of foreign

 © The Author(s) 2023. Published by Higher Education Press

transgenes or gene editing, but the design and construction of predictable novel circuits, new metabolic pathways, biological sensors, and physiological traits to fulfill the evolving needs [3–5]. Despite the difficulties in engineering the complexity of eukaryotic systems compared to prokaryotes, versatile genetic circuits can be achievable in the synthetic biology of plants. By introducing a set of genes into engineered plants, multiple plant systems have been successfully utilized to produce value-added metabolites and therapeutic proteins, such as taxadiene, L-DOPA, and vaccine of SARS-CoV-2 [6–9]. Engineered rice with a high content of essential nutrients, such as GABA, β-carotene, or anthocyanins, have the potential to solve the nutritional deficiency [10,11]. Optogenetically regulated controllers have been achieved in plants, such as the red light-controlled switch based on phytochrome B (PhyB) and the green-light-responsive system based on CarH [12,13]. A set of synthetic hormone activated Cas9-based repressors (HACRs) was introduced into *Arabidopsis thaliana* to track the levels of hormones, such as auxin, gibberellins, and jasmonates [14]. The reversible memory switch can invert the activated/deactivated states of target genes through the inversion of promoter sequences dependent on the site-specific recombinases using the phiC31 integrase and its cognate proteins [15,16]. Complete logic gates provide new possibilities for programmable manipulation of previously unrealized traits [17,18].

To some degree, these groundbreaking advancements have enabled the artificial manipulation of synthetic systems in space-time. On the other hand, there is a delay in the control of protein dosages, which is involved with the output trait in regulatory networks. The dosage imbalance between the expression of B- and C-class genes triggered by a cis-regulatory change contributed to the transformation in stamen morphology [19]. The mutation of TIL reduces the amplitude of the root clock, a temporal series of oscillating changes in gene expression, and effect the lateral root branching [20]. A HACR targeting the auxin transporter PIN-FORMED1 (PIN1) can even change the levels of the relationship between PIN1 and auxin and reduce the number of branches [14]. In addition to the expression levels, different spatial patterns were generated by combining the activities of input promoters using logic gates in *A. thaliana* roots [18].

In this manuscript, we consider the current progress in genetic parts and genetic circuits in terms of quantitative characterization and standardization, and we also highlight key challenges facing the advance of quantitative prediction, design, and application in plants.

QUANTITATIVE PLANT SYNTHETIC BIOLOGY IS EMERGING

Surging genetic parts from big data in plant science

Genetic parts are footstones for synthetic biology design with programmed purposes. The functional identification and standardized assembly are prerequisites for plant synthetic biology. Genetic parts can be mined from both prokaryotic and eukaryotic cells. Sequenced plant genome information can be fully utilized to predict available DNA parts, and the number of available DNA parts is increasing. At present, several existing genetic part libraries have been created (Table 1).

The genetic parts in these databases are mainly derived from model plants. For example, PlantProm DB contains 7723 predicted promoters by mapping full-length cDNAs on the genome of *Arabidopsis* and rice. Promoters collected in EPDNew are derived from *Arabidopsis* and maize [22]. Recently, a published web server, TDTHub, contains pre-processed data for 40 plant species, enabling more quick and intuitive research for transcriptional regulatory networks [23].

Genetic information on model plants has been widely used in the in-depth mining of bioparts. Many toolkits have also been established aiming to mine the novel genetic parts. TSSP-TCM is the first predictive tool that has been trained and adapted for promoters [35]. The transductive confidence machine (TCM) provides confidence in the predictions for each individual example in the test set. 35 promoters can be correctly predicted in a set of 40 TATA promoters; besides, 21 promoters can be predicted in a set of 25 TATA-less promoters. These regulatory element databases in turn facilitate the development of predictive tools in plants (Table 1). Based on large promoter collections from PPDB and PlantProm, TSSPlant has been developed and achieves significantly high accuracy in prediction [36].

CREs in plant pathogens promoters were revealed through the identification of *A. thaliana* transcription factor binding sites. Adjustments of the identity, density and position of these CREs can regulate the relative expression of output genes [37]. In recent years, a mass of genomic information about various plants has been revealed [21,38–40]. It means that there are extremely large amounts of potential bioparts need to be further tapped. And also, toolkits have been established aiming to mine the novel genetic parts. Three water-deficit and one salt-stress candidate genetic elements from hybrid poplar transcriptomes were used to design synthetic promoters, showing an outperformed endogenous promoter in transgenic plants [41]. 49,802,023 transposable

© The Author(s) 2023. Published by Higher Education Press

Table 1 Databases and tools for plant regulatory elements

Databases and tools	Description	Refs.
Scientific database	Multiple databases of multi-omics	
MPOD	Multi-omics database for medicinal plants	[21]
EPD	The eukaryotic promoter database	[22]
TDTHub	A web server tool for the analysis of transcription factor binding sited in plants	[23]
PPDB	A web-based plant promoter database	[24]
PlantCARE	A database of plant cis-acting regulatory elements, enhancers and repressors	[25]
OptoBase	An online platform for molecular optogenetics	[26]
TRANSFAC	A database on eukaryotic transcription factors	[27]
RGPDB	A database of root-associated genes and promoters in maize, soybean, and sorghum	[28]
TSSFinder	A tool for prediction and annotation of different organisms	[29]
PlantProm	A database of plant promoter sequences	[30]
TSSP	Prediction of plant promoters	[31]
NSITE-PL	Recognition of plant regulatory motifs	[32]
AGRIS	The *Arabidopsis* gene regulatory information server	[33]
AthaMap	A genome-wide map of putative transcription factor binding sites in *Arabidopsis thaliana*	[34]

elements (TE) records from 67 plant genomes were retrieved, and there are 62.85% retrotransposons and 37.15% DNA transposons for all known TEs [42].

The potential number of bioparts available for plant circuits is virtually unlimited. After standardization and characterization, more practical and powerful circuits can be designed with these potential bioparts.

Standardized assembly

Well-characterized and standardized building parts enable efficient engineering of versatile synthetic bio-circuits [43]. Several individual parts (promoters, localization peptides, terminators, UTRs, CDS) can be selected and assembled to produce circuits designed with predictable functions [44,45]. Such circuits in plants include toggle switches, positive feedback circuits, and logic gates to form complex functions. The DNA fragments allow different sequences with the same basic utility to be exactly exchanged with larger designs, enabling any difference in function conferred by sequence variations between parts to be functionally quantified.

With the aim of facilitating the interoperable exchange, a standardized producer for DNA fragments assembly was established for defining DNA parts. The first widely adopted standard is BioBrick [46,47]. BioBrick simplified the assembly of DNA using iterative, pairwise assembly of standardized parts (Fig. 1A). New methods for simultaneous assembly of multiple DNA fragments are more popular in plants. The Multisite Gateway method utilizes site-specific recombination based on *att*L and *att*R sites (Fig. 1B) [48,49]. A plant molecular biology toolbox with basic versatile genetic elements in the MultiSite Gateway framework was created by the Department of Plant Systems Biology (Ghent, Belgium) [50]. A cellular resolution can be achieved with cell type-specific promoters which have been cloned into Multisite Gateway-compatible entry vectors in different root cell types [51]. According to the features of Type IIS restriction enzymes the Golden Gate system allows seamless cloning through careful design of the restriction sites (Fig. 1C) [52]. The UK Biotechnological and Biological Sciences Research Council (BBSRC) defined the standard syntax based on the Golden Gate cloning method and established a common grammar for plant synthetic biology (Fig. 1D) [53]. The convenience of plug-and-play comes from the standard libraries [54–56]. Nevertheless, it is still a heavy workload to define two ends and remove conflicting intrinsic recognition sites of elements in the library, largely depending on the laboratory accumulation [56–60]. Although some toolkits are available, they are far from adequate in plant synthetic biology [54]. Additionally, the sticky ends, which have been predefined, will introduce unnecessary 1 bp or 4 bp traces into the connection of adjacent elements in the constructions.

Gene stacking using a polycistronic strategy in a single construct for the introduction of multiple genes into plants is required for manipulating complex traits and pathways. Many efficient technologies have been developed in plants based on the superposition of modular cloning systems with predefined standardization. Multiple cloning sites (MCS) were used in combination with MultiSite Gateway technology to assemble

© The Author(s) 2023. Published by Higher Education Press

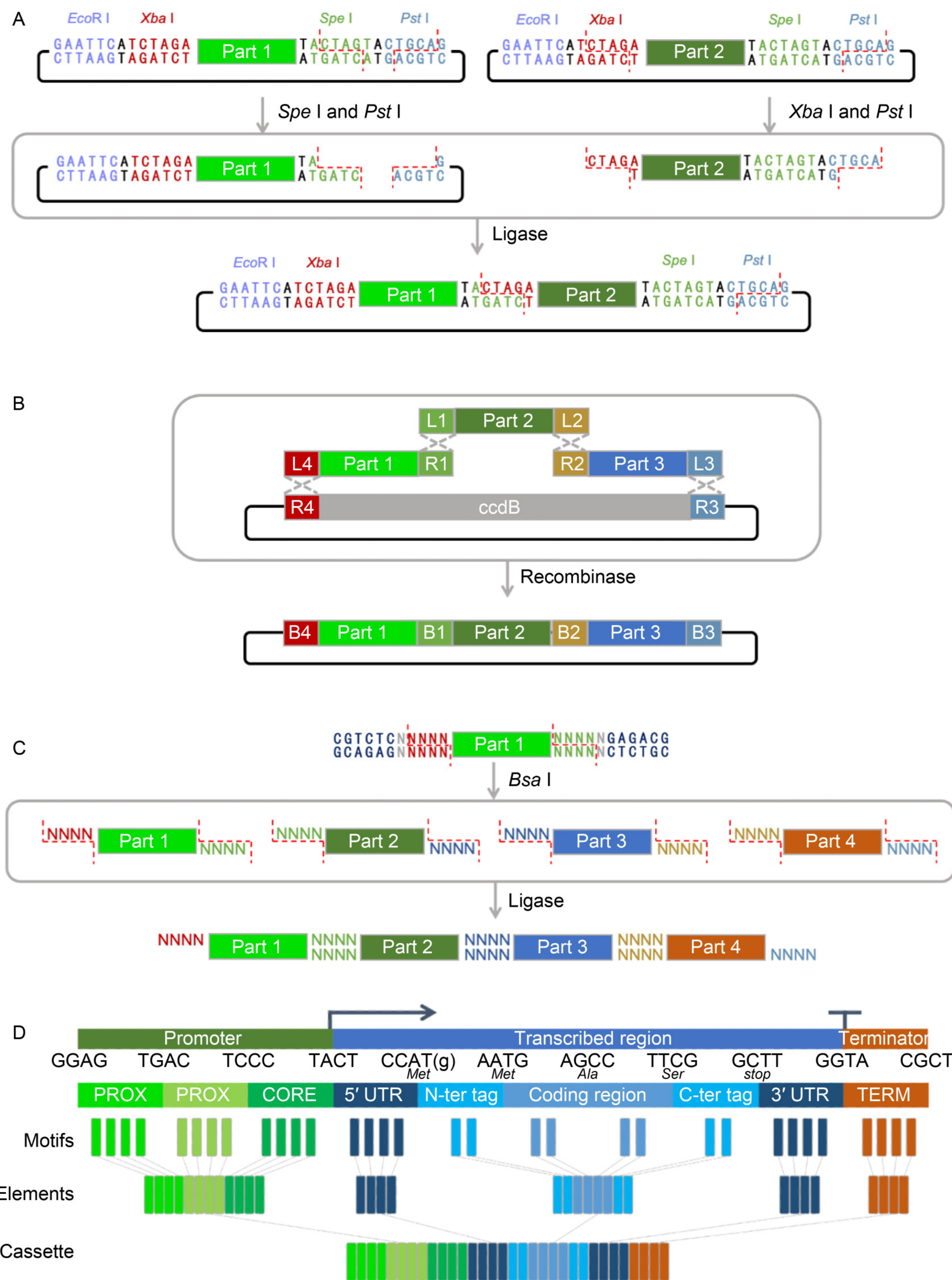

Figure 1. Schematic diagram of DNA standardized assembly principles. (A) Schematic diagram of BioBricks. Sequences are flanked by a pair of the recognition sequence of isocaudomers: *Spe* I and *Xba* I. They have the same sticky ends and the original cleavage sites will disappear after successful assembly. The new fragment combining Part 1 and Part 2 is flanked by the same recognition sequence as the original sequence. (B) Schematic diagram of Multisite Gateway. (C) Schematic diagram of GoldenBraid. Sequences flanked by a pair of the recognition sequence of the Type IIS restriction enzyme can generate compatible overhangs by digesting. Different genetic parts can be linked together in a predetermined order with T4 DNA ligase. (D) The junction points of different compositions have been defined. The elements containing varied motifs are combined by one-pot to construct a cassette in a plasmid backbone for plant transformation.

© The Author(s) 2023. Published by Higher Education Press

multiple gene cassettes [61]. Building on the standard Type IIS syntax, jStack leverages *in vivo* yeast homologous recombination to assemble large DNA fragments, such as multiple gene expression cassettes, into plant transformation vectors by one-pot [62]. TransGene Stacking II (TGSII) using improved Cre/*loxP*-mediated recombination and a transformation-competent artificial chromosome (TAC)-based acceptor vector, which is capable of large-size (>100 kb) DNA fragments [63–65]. Up to 10 gene cassettes in a single T-DNA were successfully transferred into rice [64]. Unique nucleotide sequence-guided nicking endonuclease (UNiE)-mediated DNA assembly (UNiEDA) was introduced into TGSII system and developed TGSII-UNiE to assemble more genes in parallel into acceptor/donor vectors [66]. 11 genes with three different synthetic pathways were successfully assembled into a T-DNA region with the TGSII-UNiE approach [66].

Overall, standardization is considered to be a facilitating factor in the field of plant synthetic biology. It extensively promotes genetic design and construction with engineering principles, enabling high-throughput, quantitative characterization, and large-scale constructions and comparisons of synthetic bioparts and circuits. A more traceless and standardized synthetic platform is desired.

Quantitative characterization of genetic parts

Types of genetic parts include promoters, 5′ and 3′ UTRs, protein coding sequences, terminators, activators, transcription factors, and simple regulator elements etc. The genetic parts are assembled with a fluorescent protein encoding gene to form a simple circuit, which is further transferred into the plant cells for qualitative and quantitative characterization. The biochemical parameters, that can be measured for each part, include binding affinities, transcriptional rate, quantity of repressor or activator, promoter strength, rate of protein synthesis, and RNA or protein degradation rate.

Several DNA delivery approaches, including particle bombardment, *Agrobacterium* infiltration, and protoplast transformation, allow rapid evaluation of the behaviors of introduced circuits in plant [67]. The unavoidable noise with using fluorescent proteins in plant cells is that chlorophyll may overwhelm the output signal. Therefore, ratiometric dual reporter systems, for example, firefly luciferase/*Renilla* luciferase and E-Luc/Red-F, have been developed and widely applicated in quantitative evaluation of genetic parts [55,68,69]. Self-transcribing active regulatory region sequencing (STARR-seq) has also been used for the in-depth characterization of enhancers and core promoters (Fig. 2B) [70–73]. By constructing STARR libraries with unique barcodes, up to 30,000 or even more transcriptional strength of core promoters can be obtained through a single sequencing, avoiding errors caused by multiple transformations and detections [72].

Whole plants take a long time for stable transformation, while transient expression in plant cells or protoplasts serves as a proxy. There have been several remarkable studies aiming at large-scale characterization of plant bioparts in transiently transformed *Nicotiana benthamiana* leaves [56], BY2 suspension cells [74], *Arabidopsis* protoplasts, sorghum protoplasts [68,75], and lima bean cotyledons [76]. However, the characterizations mainly focus on specific cells or tissues of these widely used model plants with an effective convenient genetic transformation system. It is known that genetic parts do not behave consistently in different plants, even in different cells of a particular species due to cell/tissue-specific or environmentally regulated expression of transacting factors and epigenetic regulators [74]. The outputs of synthetic circuits need to be carefully evaluated, because the performance of the building blocks may be varied in testing systems.

Therefore, in addition to the characterization of these elements, redesign new elements that can meet specific requirements is also an indispensable approach. Recent studies have also shown the potential for fine-tuning transgene expression levels by regulatory elements while spatiotemporal specificity is preserved [37,72,77–79]. In order to achieve quantitative expression at various levels, a rational design by combining multiple regulatory elements from various species, such as the core promoters, enhancers, 5′UTR, 3′UTR, and introns, was used to construct expression cassettes with quantitative intensities in multiple species and tissues [54,57,68,80,81]. The theoretical transcriptional activity (TTA) of cassettes with different promoter/terminator combinations is coincident with the experimental activity values in transiently transformed *Nicotiana* leaves [56].

Computer tools accelerate the processing and modeling of plant bioparts, including their structures and quantitative activities. They enable the rational design of new bioparts with a quantitative prediction (Fig. 3). Machine learning (ML) algorithms, such as support vector machine (SVM) or neural networks, greatly facilitate the qualitative/quantitative prediction and *de novo* design of bioparts. Sequence motifs identified by POsition-sensitive WoRd Set (POWERS) algorithm are used to generate novel elements, whose property of expression is comparable to plant native elements across multiple tissues and developmental stages [81,82]. Another way to obtain customizable elements is *de novo* design. Generation of random sequences with key

 © The Author(s) 2023. Published by Higher Education Press

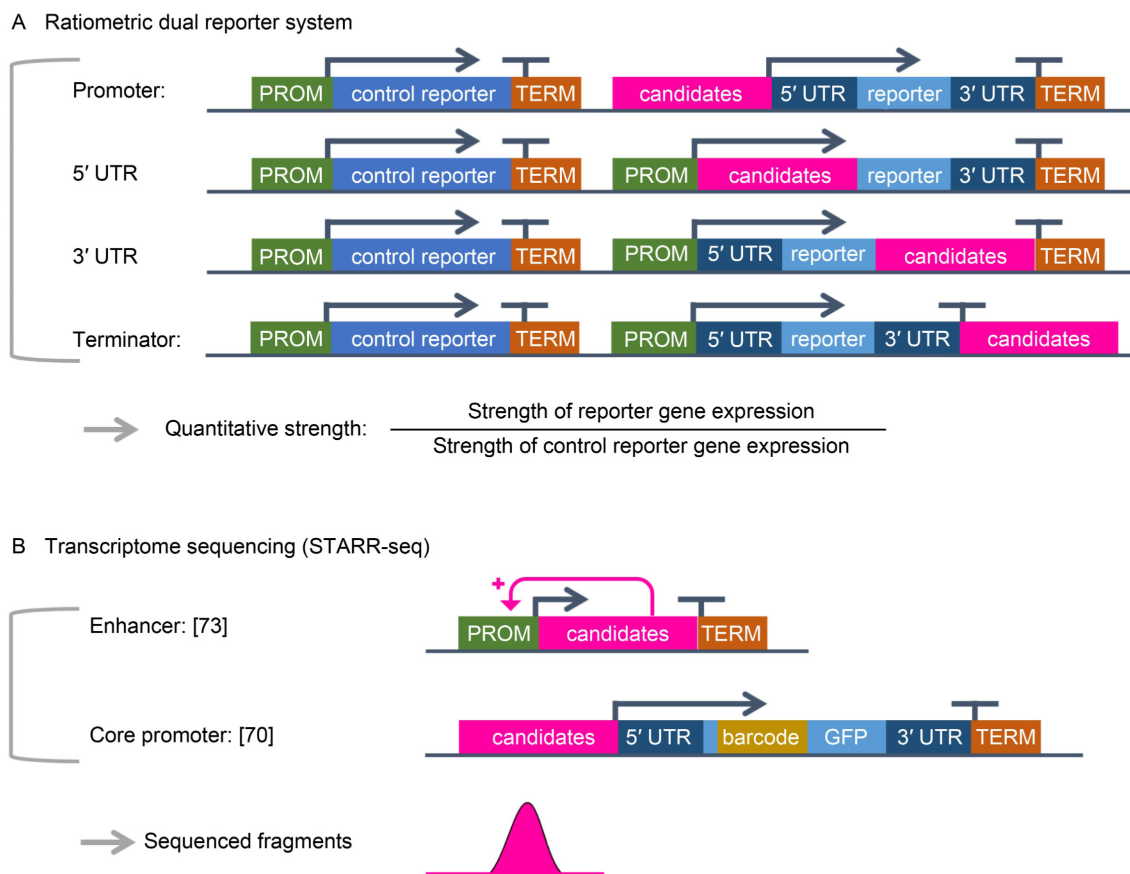

Figure 2. Schematic diagram of quantitative methods of various genetic parts. (A) The strength of candidate elements is represented by the ratio of expression level of two marker genes with dual reporter systems. The relative strength of promoters, untranslated regions (5′UTR, 3′UTR), and terminators are characterized with dual reporter systems. (B) Transcriptome sequencing can also provide the strength of elements. STARR-seq has been used to quantitatively characterize the strength of enhancers and core promoters.

features of native short plant core promoters that exhibit similar function and transcriptional intensity in *Arabidopsis* and maize [72]. A convolutional neural network (CNN) model, which can predict the strength of core promoters, was further used for *in silico* evolution and a large increase in strength was observed after three rounds [72]. Based on the detailed and sufficient characterization of the core promoter both in sequences and intensity by transcriptome sequencing approach, these synthetic elements were successfully constructed. The comprehensive understanding of plant regulatory elements will lead to the flexibility and controllability in genetic circuit design [83].

Quantitative artificial circuits

By introducing multi-genes, many value-added metabolites have been successfully produced in different plants (Table 2). Multi-gene transformation is the inevitable trend of modern synthetic biology. Diverse regulatory elements and regulatory systems have been used in plants [13,16,98]. However, when introducing multiple genes, the elements in practical applications are actually limited and the number has begun to flatten out. Hence, developing new elements and new modes of expression control with specific quantities is of great significance. Quantitative expression of genes is decisive and how to design complex synthetic systems with quantitative predictions with different purposes in plants will be a long-term research topic in the future. Transposons-mediated and signal-response synthetic switches have been developed and applied to manipulate the physiological processes and metabolic pathways in plants [99–101]. Many valuable synthetic tools have also been developed in plants, such as reversible memory switches, optogenetic switches, and logic gates [16,17,102].

The clustered regularly interspaced short palindromic repeats (CRISPR) systems have been successfully used to manipulate gene knockout/knock-in/knockdown in

© The Author(s) 2023. Published by Higher Education Press

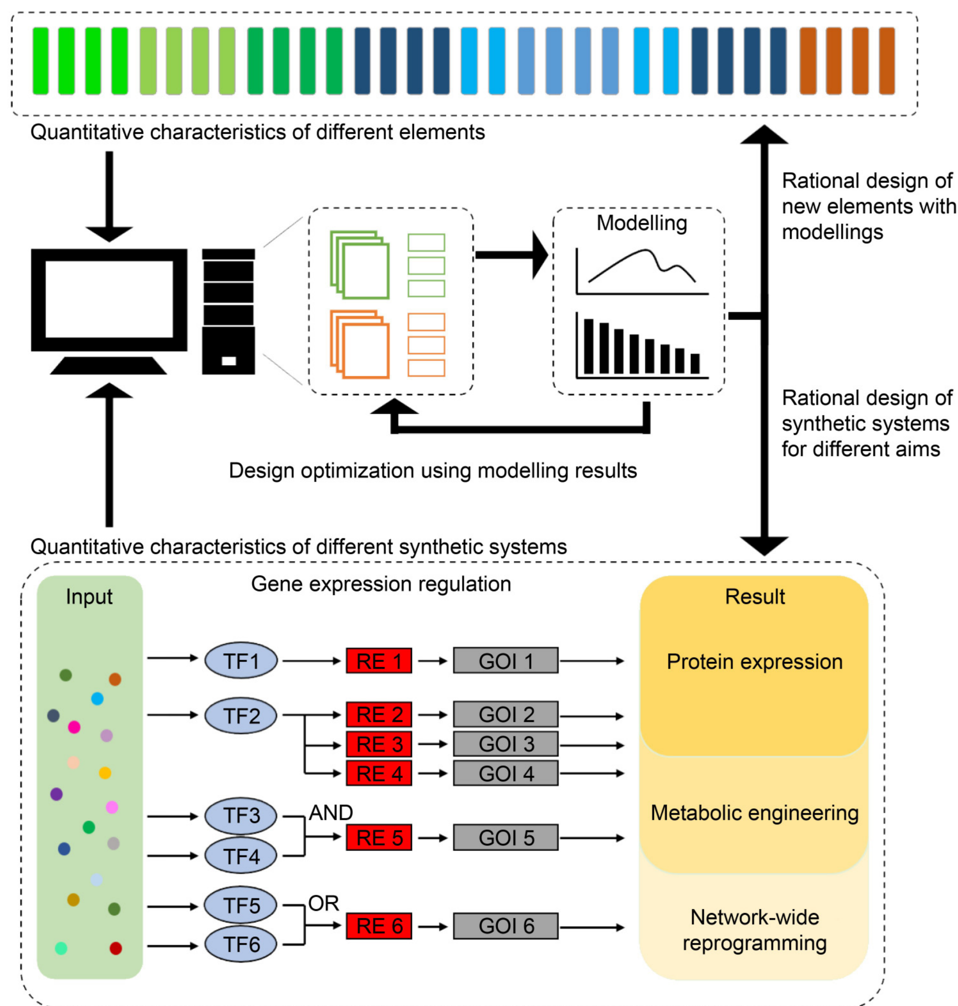

Figure 3. Diagrammatic representation of rational design with computer tools. Elements with quantitative and comprehensive characterization are analyzed with computer tools. The corresponding models built beyond the information are used to create new elements with quantitative predictions and can guide the construction of synthetic systems with different purposes.

plants. CRISPR-Cas9 system has been used to engineer quantitative variation for yield-related traits in maize by editing *CLE* promoter sequences [103]. Cis-regulatory variation and promoter engineering have also been used to improve tomato and rice properties [104,105].

Synthetic transcriptional repressors or activators based on nuclease-inactivated CRISPR/dCas are increasingly popular for their high accuracy and multiplexing capacity, providing new strategies for designing synthetic gene circuits [106,107]. A simple CRISPR activator, which contains a single VP64 domain fusion to dCas9, shows low activation levels [108]. Effective strategies from the genetic engineering of microbes and mammalian cells are developed to improve the capacities in plant synthetic biology [109–112]. Some studies focus on the engineering of dCas by fusing a Suntag, a tripartite complex VPR, EDLL, TV, and so on [113,114]. A few studies focus on the modification of gRNA or crRNA, such as SAM and scaffold RNA [111,115]. A comprehensive, quantitative, and systematic comparison of these designs was reported in *N. benthamiana* [115]. A variant of the scRNA-gRNA2.0 scaffold (used in dCas EV2.1) with a spontaneous mutation shows the highest transcriptional activation [115]. It has been applied to re-route metabolic fluxes towards the accumulation of flavonoids with simultaneous regulation of up to six enzymes [116].

RNA scaffold is a powerful machinery coordinating the transcriptional regulation of multiple genes due to their ability in activating and repressing sets of genes simultaneously, permitting more sophisticated designs in yeast and human cells [111]. A combination system, CRISPR-Combo, permits simultaneous genome editing and gene activation in plants [117]. This suggests that

 © The Author(s) 2023. Published by Higher Education Press

Table 2 Engineering of multiple genes and the corresponding regulatory elements used in recent years

Products	Plant chassis		The number of genes	Used promoters	Used terminators	Refs.
Omega-3 long chain polyunsaturated fatty acids	*Camelina sativa* seed;	Stable transformation	9	P_{USP}, P_{CNL}, P_{SBP}, P_{NP}, P_{PvArc}, P_{GLY}, P_{CsVMV}	$T_{35S}$$T_{OCS}$,$T_{CatpA}$, T_{E9}, T_{PvArc}, T_{NOS}, T_{HSP}, $T_{Phaseolin}$, T_{Gly}	[84]
Omega-3 long chain polyunsaturated fatty acids	*Brassica napus* seed; *Brassica juncea* seed	Stable transformation	9	P_{FP1}, P_{FAE1}, P_{CNL1}, P_{CNL2}, P_{35S}	T_{NOS}, T_{Lectin}, T_{CNL1}, T_{CNL2}	[85,86]
Biomass	*Nicotiana tabacum*	Stable transformation	3	P_{NOS}, P_{35S}	T_{NOS}	[87]
CoQ_{10}	*Solanum lycopersicum* fruit	Stable transformation	5	P_{E8}, P_{35S}	T_{AtHSP}, T_{35S}	[88]
Casbene-derived diterpenoids	*Nicotiana benthamiana* leaf	Transient transformation; stable transformation	6	P_{35S}, P_{StSTLS}, P_{AtCab1}, P_{AtuNos}, P_{AtuMAS}, $P_{SlRbcS1}$, $P_{SlRbcS2}$, $P_{AtRbcS1B}$, $P_{SlRbcS3A}$, P_{SlH4}, $P_{AtLHB1B1}$	T_{AtuMAS}, T_{Atug7}, T_{AtuOCS}, T_{AtuNos}	[89]
Auxin analogs	*N. benthamiana* leaf	Transient transformation	4	P_{35S}	T_{CaMV}, T_{polyA}	[90]
Astaxanthin	*N. benthamiana*	Stable transformation	2	P_{35S}; $P_{FMVm\ 34S}$	T_{NOS}, T_{35S}	[91]
Iron and zinc concentrations	*Manihot esculenta* storage root	Stable transformation	2	P_{A14}, $P_{patatin1}$	$T_{35S,}$ T_{NOS}	[92]
Folate	*Z. mays* and *Triticum aestivum* endosperm	Stable transformation	3	P_{35S}, P_{Leg1A}, P_{GluC}	T_{35S}, T_{NOS}	[93]
Astaxanthin	*Zea mays* seed	Stable transformation	9	P_{35S}, P_{2BDEN} (P_{R5SGPA}/$P_{2R5SGPA}$)	T_{35S}, T_{NOS}	[94]
N-formyldemecolcine	*N. benthamiana* leaf	Transient transformation	16	P_{35S}	T_{NOS}	[95]
Taxadiene-5*α*-ol	*N. benthamiana* leaf	Transient transformation	4	P_{35S}	T_{NOS}	[96]
Pheromone	*N. benthamiana*	Stable transformation	4	P_{35S}	T_{35S}	[97]
Violacein	*N. benthamiana* leaf	Transient transformation	5	$P_{AtHsp18}$, P_{MAS}, P_{Act2}, P_{RbcS}, P_{Bch1}	T_{AtHsp}, T_{AtRbcS}, T_{AtAct2}, T_{AtUbq3}, T_{NOS}	[62]
Astaxanthin	Rice endosperm	Stable transformation	4	P_{Glub1}, P_{Glub4}, P_{Glb1}, P_{GluC}	T_{mas}, T_{ags}, T_{35S}	[63]
Anthocyanins	Rice endosperm	Stable transformation	8	P_{10KDa}, P_{16KDa}, P_{npr33}, P_{Glub5}, P_{Glb1}, P_{Glub4}, P_{Glub1}, P_{GluC}	T_{rbc}, T_{mas}, T_{NOS}, T_{ocs}, T_{ags}, T_{35S}	[64]

achieving the complex multilayered regulation in plants is promising.

THE CHALLENGES FOR QUANTITATIVE SYNTHETIC BIOLOGY IN PLANTS

Considering the natural characteristics of plant cells of multi-organelle and multi-tissue, it is intractable to construct multilayered synthetic systems in plants. There are several current challenges for quantitative plant synthetic biology.

Detailed quantitative descriptions on genetic parts are limited

The limited understanding of plant genetic components restricts the application and upgrade of potential bioparts in plants. Relying on pre-existing knowledge of CREs, more efficient and more diverse synthetic biology parts can be obtained through the mutation, rearrangement, and combination of fragments or motifs in promoters. The engineering of synthetic circuits remains heavily dependent on the interaction of specific CREs and transcription factors [118–120]. However, the current understanding of transcription factors is still insufficient, potentially millions of TF-CRE interactions need to be validated even in *Arabidopsis* genome [121]. Besides, not all functionalities of CREs are clearly performed. It has been shown that some core promoters display enhancer activity, while some enhancers also potentially carry sequence motifs of core promoters such as TATA-box [73,122]. Therefore, although a number of regulatory elements are predicable from genomic information with the assist of genetic tools, their functions still need to be experimentally tested. In addition to the function of elements, the unclear mechanism of distal regulatory elements has severely hindered their applications. Enhancers and silencers

© The Author(s) 2023. Published by Higher Education Press

affect gene expressions through chromatin interactions, and in some case the distances of these regulatory sequences can be several dozens of megabases [123]. 34% of enhancers potentially regulate multi-genes and most of them target immediately flanking genes, skipping at least one gene [124]. The complexity of distal regulatory elements is an important reason that few of them were applied in the rational design of synthetic promoters. Poor analysis of these element sequences in plants, especially the CREs, hinders their reconstruction. Elucidation of these plant genetic components can provide useful information for functional design.

Genetic parts with biochemical parameters have been well-characterized and used to produce circuits in single-celled microorganisms, including promoter strengths, transcriptional rates, translation rates, quantity of repressors/activators, binding affinities, and protein degradation rates [125–129]. Artificial genetic circuits can be rationally designed with a software tool, Cello, in *Escherichia coli* and in *Saccharomyces cerevisiae* [130,131]. Previous synthetic biology research has demonstrated that even if the function of each element is known, it is still probably out of work as expected [132]. Only 4 of 10 synthetic repressors decreased the expression level of green fluorescent protein (GFP) and none achieved complete repression [18]. Except for the functional properties, a quantitative understanding of these CREs and TF-CRE interactions are also critical to select the optimal parts to assemble into functional devices with quantitatively predictable functions. The contradiction between a large number of element resources and a small number of applications stems from inadequate quantitative annotation. The DNA sequences of output promoters and their ON/OFF signal strength in standardized units were necessary in designing artificial synthetic circuits in *E. coli* (Fig. 4). The quality of the mathematical models describing the gate is central of these genetic design automation software [133,134]. Quantitative characterization of plant bioparts lags that of prokaryotes. As we discussed above, the dual-luciferases systems, a mathematical model, and barcode tagging-based sequencing were used to accurately characterize individual parts in *N. benthamiana* leaves and plant protoplasts with transient assays. The quantitative data will not tell us about the performance of these parts in other-type untested cells or in a stably transformed state. Novel high-throughput biopart characterization techniques in multiple-type cells are underexplored.

Genetic parts should be orthogonal

Many plant bioparts are not orthogonal enough across chassis cells. Orthogonality means the bioparts can function independently of the host and other bioparts, consequently ensuring predictability. The established construction may be crashed because of this consequent variability. The regulatory networks vary widely in individual plant cells. Some plant-derived promoters have inconsistent performances for gene expression in *N. benthamiana* leaves and BY2 suspension cells [74]. Refactoring is a process in which the genetic part's native design parameters are simplified, and inefficiencies and endogenous regulation are removed, while still retaining the essential function of the part [83]. The position effect and the interference of the surrounding sequence can also lead to variability. Insulators can mitigate this effect through the modulation of chromatin structure, but these elements work differently in different plant cells [135]. How to build synthetic circuits as desired and achieve complete orthogonality between the system and the host chassis remains to be further developed.

Building capacity is limited

As discussed above, various standardized large fragments assembly methods have been applied in plants. Different standardizations have been combined at different levels to realize the design and assembly of more diversity and longer sequences. In addition to the genetic constructs, genomic sequence re-editing is also an essential step in achieving synthetic regulation. Rapid progress has been made in the development of base editing, gene targeting, and gene insertion. CRISPR-associated nucleases play a critical role in genomic reprogramming [136,137]. It is feasible to simultaneously edit multiple target genes and modulate gene expressions. Yeast-mediated modification of the chloroplast genome of the green alga, *Chlamydomonas reinhardtii*, demonstrates a new approach to modify the chloroplast genome [136,137]. Cleavage-free genomic engineering approaches of large sequences still have a lot of development potential. There are still no reports about synthetic chromosomes in plants, while single-chromosome yeast has been successfully created [138].

Genetic compiler is used to predict the behaviors of genetic circuits

Genetic compiler is a system that allows computers and robotics to select and assemble the optimal parts for a specified behavior of a genetic circuit. Digital quantitative descriptions of parts and circuits will provide credible information to reduce the laborious process of trial-and-error. Biological processes can be described as complex networks of signaling pathways

 © The Author(s) 2023. Published by Higher Education Press

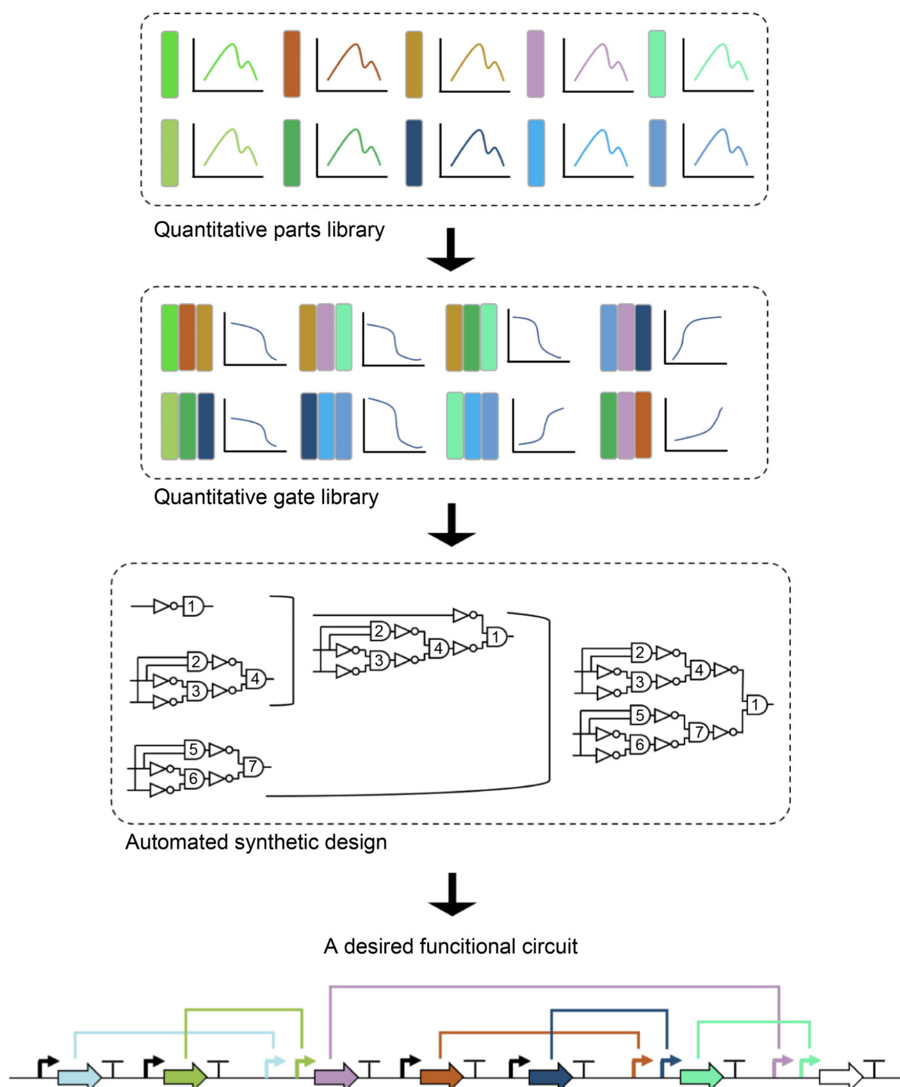

Figure 4. An example of an automated design-construct module.

and translated into mathematical models for computer simulation [139]. An accurate mathematical model enables complex computational control by synthetic circuits.

Whole-cell models of *Mycoplasma genitalium* and *E. coli* have been published [140–142]. As an important eukaryotic model organism, more than 14 versions of *S. cerevisiae* genome-scale metabolic models have been created [143]. The Virtual Physiological Rat (VPR) projects have made significant progress in predictable medicine [144].

Compared to these advances, research in plant cells still lags far behind. Flourishing plant omics data collected from plant tissues or cells provide gene expression, metabolite formation, and protein biochemical information. Many robust models have been developed in plants, such as the C3 and C4 photosynthetic process, flowering, gene regulatory network, and root growth [145,146]. There is a vacancy of multi-scale mathematical models for quantitative description of plant regulatory networks, even of the most used model plant, *A. thaliana*.

CONCLUSION

Synthetic biology has made rapid progresses in microbial chassis in recent years. Many biological activities or intercellular signals of microorganisms and human cells have already been described and fine-tuned by mathematical models [147–150]. Quantitative models of input-output relationships have been used to design multicellular circuits in yeast. Computational predictions with high predictability and reliability have been tracked by experiments [151]. Compared to the above, the quantitative research in plants is still in its infant stage, confined to a single group of quiescent cells, with little

© The Author(s) 2023. Published by Higher Education Press

involvement in cellular dynamics. Therefore, plant synthetic biology researchers can learn from these extraordinary works on microorganisms and human cells.

With the consideration of plant species and different tissues, the differences between plant cells are greater than those of microorganisms. More tissue- and condition-specific element resources are required. The hottest technical, single-cell sequencing (SCS), is a promising approach to explore potential elements with distinct patterns [152–155]. Cell-specific regulatory elements can be identified by comparing the data from different single-cells.

In addition, plants are sensitive to the environment. Light, temperature, and air quality can affect the expected performance of synthetic gene circuits. These various variables should be considered in the relevant mathematical models, which hinder the development of real-time quantitative monitoring methods and tools for plant elements and logic circuits. For complex regulatory systems, especially some metabolic networks and multicellularity interactions, how to accurately describe the functional status and properties of synthetic systems should be taken into consideration.

Plant synthetic biology is an important approach that can provide solutions to tackle many problems in field of agriculture, health care, and energy resources in the future. In the process of its application and commercialization, quantitative engineering principles are required, which can guarantee some more practical and robust systems in plants. In conclusion, the limited knowledge of plant elements, the low compatibility of toolkits, and the backwardness of quantitative functional characterization are the most important challenges in plant synthetic biology. These challenges should be emphasized in further research.

ACKNOWLEDGEMENTS

This work was supported by the National Key Research and Development Program of China (No. 2018YFA0900600), the Strategic Priority Research Program "Molecular mechanism of Plant Growth and Development" of Chinese Academy of Science (No. XDB27020202), the National Natural Science Foundation of China (Nos. 22077129, 32070328 and 41876084), the Natural Science Foundation of Shanghai Municipal Science and Technology Committee (No. 21ZR1470900), the Tianjin Synthetic Biotechnology Innovation Capacity Improvement Project (No. TSBICIP-KJGG-002-15), the Program of Shanghai Academic Research Leader (No. 20XD1404400). This work was also financially supported by the Construction of the Registry and Database of Bioparts for Synthetic Biology of the Chinese Academy of Science (No. ZSYS-016), the International Partnership Program of Chinese Academy of Science (No. 153D31KYSB20170121), and the National Key Laboratory of Plant Molecular Genetics, SIPPE, Chinese Academy of Science.

COMPLIANCE WITH ETHICS GUIDELINES

Conflicts of interest The authors Chenfei Tian, Jianhua Li, and Yong Wang declare that they have no conflict of interests.

This article is a review and does not contain any human or animal subjects performed by any of the authors.

OPEN ACCESS

This article is licensed by the CC By under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

REFERENCES

1. Endy, D. (2005) Foundations for engineering biology. Nature, 438, 449−453
2. Zhang, L., Chang, S. and Wang, J. (2011) Synthetic biology: from the first synthetic cell to see its current situation and future development. Chin. Sci. Bull., 56, 229−237
3. Fausther-Bovendo, H. and Kobinger, G. (2021) Plant-made vaccines and therapeutics. Science, 373, 740−741
4. Zhu, X., Liu, X., Liu, T., Wang, Y., Ahmed, N., Li, Z. and Jiang, H. (2021) Synthetic biology of plant natural products: from pathway elucidation to engineered biosynthesis in plant cells. Plant Commun., 2, 100229
5. Jiao, Y., Han, Y., Yang, Q., Huang, Y., An, J., Yang, Y. and Ye, J. (2021) Commercialization development trend of genetically modified maize and the enlightenment. Shengwu Jishu Tongbao (in Chinese), 37, 164−176
6. Imamura, T., Isozumi, N., Higashimura, Y., Ohki, S. and Mori, M. (2021) Production of ORF8 protein from SARS-CoV-2 using an inducible virus-mediated expression system in suspension-cultured tobacco BY-2 cells. Plant Cell Rep., 40, 433−436
7. Diego-Martin, B., González, B., Vazquez-Vilar, M., Selma, S., Mateos-Fernández, R., Gianoglio, S., Fernández-Del-Carmen, A. and Orzáez, D. (2020) Pilot production of SARS-CoV-2 related proteins in plants: a proof of concept for rapid repurposing of indoor farms into biomanufacturing facilities. Front. Plant Sci., 11, 612781
8. Breitel, D., Brett, P., Alseekh, S., Fernie, A. R., Butelli, E. and Martin, C. (2021) Metabolic engineering of tomato fruit enriched in L-DOPA. Metab. Eng., 65, 185−196
9. Molina-Hidalgo, F. J., Vazquez-Vilar, M., D'Andrea, L., Demurtas, O. C., Fraser, P., Giuliano, G., Bock, R., Orzáez, D.

© The Author(s) 2023. Published by Higher Education Press

and Goossens, A. (2021) Engineering metabolism in nicotiana species: a promising future. Trends Biotechnol., 39, 901−913
10. Akama, K., Kanetou, J., Shimosaki, S., Kawakami, K., Tsuchikura, S. and Takaiwa, F. (2009) Seed-specific expression of truncated *OsGAD2* produces GABA-enriched rice grains that influence a decrease in blood pressure in spontaneously hypertensive rats. Transgenic Res., 18, 865−876
11. Zhu, Q., Wang, B., Tan, J., Liu, T., Li, L. and Liu, Y. G. (2019) Plant synthetic metabolic engineering for enhancing crop nutritional quality. Plant Commun., 1, 100017
12. Müller, K., Siegel, D., Rodriguez Jahnke, F., Gerrer, K., Wend, S., Decker, E. L., Reski, R., Weber, W. and Zurbriggen, M. D. (2014) A red light-controlled synthetic gene expression switch for plant systems. Mol. Biosyst., 10, 1679−1688
13. Chatelle, C., Ochoa-Fernandez, R., Engesser, R., Schneider, N., Beyer, H. M., Jones, A. R., Timmer, J., Zurbriggen, M. D. and Weber, W. (2018) A green-light-responsive system for the control of transgene expression in mammalian and plant cells. ACS Synth. Biol., 7, 1349−1358
14. Khakhar, A., Leydon, A. R., Lemmex, A. C., Klavins, E. and Nemhauser, J. L. (2018) Synthetic hormone-responsive transcription factors can monitor and re-program plant development. eLife, 7, e34702
15. Gomide, M. S., Sales, T. T., Barros, L. R. C., Limia, C. G., de Oliveira, M. A., Florentino, L. H., Barros, L. M. G., Robledo, M. L., José, G. P. C., Almeida, M. S. M., *et al*. (2020) Genetic switches designed for eukaryotic cells and controlled by serine integrases. Commun. Biol., 3, 255
16. Bernabé-Orts, J. M., Quijano-Rubio, A., Vazquez-Vilar, M., Mancheño-Bonillo, J., Moles-Casas, V., Selma, S., Gianoglio, S., Granell, A. and Orzaez, D. (2020) A memory switch for plant synthetic biology based on the phage ϕC31 integration system. Nucleic Acids Res., 48, 3379−3394
17. Lloyd, J. P. B., Ly, F., Gong, P., Pflueger, J., Swain, T., Pflueger, C., Fourie, E., Khan, M. A., Kidd, B. N. and Lister, R. (2022) Synthetic memory circuits for stable cell reprogramming in plants. Nat. Biotechnol., 40, 1862−1872
18. Brophy, J. A. N., Magallon, K. J., Duan, L., Zhong, V., Ramachandran, P., Kniazev, K. and Dinneny, J. R. (2022) Synthetic genetic circuits as a means of reprogramming plant roots. Science, 377, 747−751
19. Liu, J., Li, C. Q., Dong, Y., Yang, X. and Wang, Y. Z. (2018) Dosage imbalance of B- and C-class genes causes petaloid-stamen relating to F_1 hybrid variation. BMC Plant Biol., 18, 341
20. Dickinson, A. J., Zhang, J., Luciano, M., Wachsman, G., Sandoval, E., Schnermann, M., Dinneny, J. R. and Benfey, P. N. (2021) A plant lipocalin promotes retinal-mediated oscillatory lateral root initiation. Science, 373, 1532−1536
21. He, S., Yang, L., Ye, S., Lin, Y., Li, X., Wang, Y., Chen, G., Liu, G., Zhao, M., Zhao, X., *et al*. (2022) MPOD: Applications of integrated multi-omics database for medicinal plants. Plant Biotechnol. J., 20, 797−799
22. Dreos, R., Ambrosini, G., Groux, R., Cavin Périer, R. and Bucher, P. (2017) The eukaryotic promoter database in its 30th year: focus on non-vertebrate organisms. Nucleic Acids Res., 45, D51−D55
23. Grau, J. and Franco-Zorrilla, J. M. (2022) TDTHub, a web server tool for the analysis of transcription factor binding sites in plants. Plant J., 111, 1203−1215
24. Kusunoki, K. and Yamamoto, Y. Y. (2017) Plant promoter database (PPDB). Methods Mol. Biol., 1533, 299−314
25. Lescot, M., Déhais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., Rouzé, P. and Rombauts, S. (2002) PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. Nucleic Acids Res., 30, 325−327
26. Kolar, K., Knobloch, C., Stork, H., Žnidarič, M. and Weber, W. (2018) OptoBase: a web platform for molecular optogenetics. ACS Synth. Biol., 7, 1825−1828
27. Matys, V., Kel-Margoulis, O. V., Fricke, E., Liebich, I., Land, S., Barre-Dirrie, A., Reuter, I., Chekmenev, D., Krull, M., Hornischer, K., *et al*. (2006) TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. Nucleic Acids Res., 34, D108−D110
28. Moisseyev, G., Park, K., Cui, A., Freitas, D., Rajagopal, D., Konda, A. R., Martin-Olenski, M., Mcham, M., Liu, K., Du, Q., *et al*. (2020) RGPDB: database of root-associated genes and promoters in maize, soybean, and sorghum. Database (Oxford), 2020, baaa038
29. de Medeiros Oliveira, M., Bonadio, I., Lie de Melo, A., Mendes Souza, G. and Durham, A. M. (2021) TSSFinder-fast and accurate *ab initio* prediction of the core promoter in eukaryotic genomes. Brief. Bioinform., 22, bbab198
30. Shahmuradov, I. A., Gammerman, A. J., Hancock, J. M., Bramley, P. M. and Solovyev, V. V. (2003) PlantProm: a database of plant promoter sequences. Nucleic Acids Res., 31, 114−117
31. Solovyev, V. V., Shahmuradov, I. A. and Salamov, A. A. (2010) Identification of promoter regions and regulatory sites. Methods Mol. Biol., 674, 57−83
32. Shahmuradov, I. A. and Solovyev, V. V. (2015) Nsite, NsiteH and NsiteM computer tools for studying transcription regulatory elements. Bioinformatics, 31, 3544−3545
33. Yilmaz, A., Mejia-Guerra, M. K., Kurz, K., Liang, X., Welch, L. and Grotewold, E. (2011) AGRIS: the *Arabidopsis* gene regulatory information server, an update. Nucleic Acids Res., 39, D1118−D1122
34. Hehl, R., Norval, L., Romanov, A. and Bülow, L. (2016) Boosting AthaMap database content with data from protein binding microarrays. Plant Cell Physiol., 57, e4
35. Shahmuradov, I. A., Solovyev, V. V. and Gammerman, A. J. (2005) Plant promoter prediction with confidence estimation. Nucleic Acids Res., 33, 1069−1076
36. Shahmuradov, I. A., Umarov, R. K. and Solovyev, V. V. (2017) TSSPlant: a new tool for prediction of plant Pol II promoters. Nucleic Acids Res., 45, gkw1353
37. Cai, Y. M., Kallam, K., Tidd, H., Gendarini, G., Salzman, A. and Patron, N. J. (2020) Rational design of minimal synthetic

© The Author(s) 2023. Published by Higher Education Press

promoters for plants. Nucleic Acids Res., 48, 11845−11856

38. Duvick, J., Fu, A., Muppirala, U., Sabharwal, M., Wilkerson, M. D., Lawrence, C. J., Lushbough, C. and Brendel, V. (2008) PlantGDB: a resource for comparative plant genomics. Nucleic Acids Res., 36, D959−D965
39. Liu, Y., Wang, Z., Wu, X., Zhu, J., Luo, H., Tian, D., Li, C., Luo, J., Zhao, W., Hao, H., *et al*. (2021) SorGSD: updating and expanding the sorghum genome science database with new contents and tools. Biotechnol. Biofuels, 14, 165
40. Chen, F., Dong, W., Zhang, J., Guo, X., Chen, J., Wang, Z., Lin, Z., Tang, H. and Zhang, L. (2018) The sequenced angiosperm genomes and genome databases. Front. Plant Sci., 9, 418
41. Yang, Y., Lee, J. H., Poindexter, M. R., Shao, Y., Liu, W., Lenaghan, S. C., Ahkami, A. H., Blumwald, E. and Stewart, C. N. Jr. (2021) Rational design and testing of abiotic stress-inducible synthetic promoters from poplar cis-regulatory elements. Plant Biotechnol. J., 19, 1354−1369
42. Pedro, D. L. F., Amorim, T. S., Varani, A., Guyot, R., Domingues, D. S. and Paschoal, A. R. (2021) An atlas of plant transposable elements. F1000 Res., 10, 1194
43. Clarke, L. J. and Kitney, R. I. (2016) Synthetic biology in the UK—An outline of plans and progress. Synth. Syst. Biotechnol., 1, 243−257
44. Chung, S. M., Frankman, E. L. and Tzfira, T. (2005) A versatile vector system for multiple gene expression in plants. Trends Plant Sci., 10, 357−361
45. Nakagawa, T., Kurose, T., Hino, T., Tanaka, K., Kawamukai, M., Niwa, Y., Toyooka, K., Matsuoka, K., Jinbo, T. and Kimura, T. (2007) Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. J. Biosci. Bioeng., 104, 34−41
46. Smolke, C. D. (2009) Building outside of the box: iGEM and the BioBricks foundation. Nat. Biotechnol., 27, 1099−1102
47. Knight, T. (2003) Idempotent Vector Design for Standard Assembly of Biobricks. Cambridge: Mit Artificial Intelligence Laboratory; Mit Synthetic Biology Working Group
48. Smedley, M. A. and Harwood, W. A. (2015) Gateway®-compatible plant transformation vectors. Methods Mol. Biol., 1223, 3−16
49. Karimi, M., Depicker, A. and Hilson, P. (2007) Recombinational cloning with plant gateway vectors. Plant Physiol., 145, 1144−1154
50. Karimi, M., Bleys, A., Vanderhaeghen, R. and Hilson, P. (2007) Building blocks for plant gene assembly. Plant Physiol., 145, 1183−1191
51. Marquès-Bueno, M. D. M., Morao, A. K., Cayrel, A., Platre, M. P., Barberon, M., Caillieux, E., Colot, V., Jaillais, Y., Roudier, F. and Vert, G. (2016) A versatile Multisite Gateway-compatible promoter and transgenic line collection for cell type-specific functional genomics in *Arabidopsis*. Plant J., 85, 320−333
52. Engler, C., Kandzia, R. and Marillonnet, S. (2008) A one pot, one step, precision cloning method with high throughput capability. PLoS One, 3, e3647
53. Patron, N. J., Orzaez, D., Marillonnet, S., Warzecha, H., Matthewman, C., Youles, M., Raitskin, O., Leveau, A., Farré, G., Rogers, C., *et al*. (2015) Standards for plant synthetic biology: a common syntax for exchange of DNA parts. New Phytol., 208, 13−19
54. Engler, C., Youles, M., Gruetzner, R., Ehnert, T. M., Werner, S., Jones, J. D., Patron, N. J. and Marillonnet, S. (2014) A golden gate modular cloning toolbox for plants. ACS Synth. Biol., 3, 839−843
55. Sarrion-Perdigones, A., Falconi, E.E., Zandalinas, S.I., Juárez, P., Fernández-del-Carmen, A., Granell, A. and Orzaez, D. (2011) GoldenBraid: an iterative cloning system for standardized assembly of reusable genetic modules. PLoS One. 6, e21622
56. Sarrion-Perdigones, A., Vazquez-Vilar, M., Palací, J., Castelijns, B., Forment, J., Ziarsolo, P., Blanca, J., Granell, A. and Orzaez, D. (2013) GoldenBraid 2. 0: a comprehensive DNA assembly framework for plant synthetic biology. Plant Physiol., 162, 1618−1631
57. Vazquez-Vilar, M., Quijano-Rubio, A., Fernandez-Del-Carmen, A., Sarrion-Perdigones, A., Ochoa-Fernandez, R., Ziarsolo, P., Blanca, J., Granell, A. and Orzaez, D. (2017) GB3.0: a platform for plant bio-design that connects functional DNA elements with associated biological data. Nucleic Acids Res., 45, 2196−2209
58. Vazquez-Vilar, M., Garcia-Carpintero, V., Selma, S., Bernabé-Orts, J. M., Sanchez-Vicente, J., Salazar-Sarasua, B., Ressa, A., de Paola, C., Ajenjo, M., Quintela, J. C., *et al*. (2021) The GB4.0 platform, an All-In-One tool for CRISPR/Cas-based multiplex genome engineering in plants. Front. Plant Sci., 12, 689937
59. Vazquez-Vilar, M., Juarez, P., Bernabé-Orts, J. M. and Orzaez, D. (2022) Design of multiplexing CRISPR/Cas9 constructs for plant genome engineering using the GoldenBraid DNA assembly standard. Methods Mol. Biol., 2379, 27−44
60. González, B., Vazquez-Vilar, M., Sánchez-Vicente, J. and Orzáez, D. (2022) Optimization of vectors and targeting strategies including GoldenBraid and genome editing tools: GoldenBraid assembly of multiplex CRISPR/Cas12a guide RNAs for gene editing in *Nicotiana benthamiana*. Methods Mol. Biol., 2480, 193−214
61. Vemanna, R. S., Chandrashekar, B. K., Hanumantha Rao, H. M., Sathyanarayanagupta, S. K., Sarangi, K. S., Nataraja, K. N. and Udayakumar, M. (2013) A modified MultiSite gateway cloning strategy for consolidation of genes in plants. Mol. Biotechnol., 53, 129−138
62. Shih, P. M., Vuu, K., Mansoori, N., Ayad, L., Louie, K. B., Bowen, B. P., Northen, T. R. and Loqué, D. (2016) A robust gene-stacking method utilizing yeast assembly for plant synthetic biology. Nat. Commun., 7, 13215
63. Zhu, Q., Zeng, D., Yu, S., Cui, C., Li, J., Li, H., Chen, J., Zhang, R., Zhao, X., Chen, L., *et al*. (2018) From golden rice to aSTARice: Bioengineering astaxanthin biosynthesis in rice endosperm. Mol. Plant, 11, 1440−1448

 © The Author(s) 2023. Published by Higher Education Press

64. Zhu, Q., Yu, S., Zeng, D., Liu, H., Wang, H., Yang, Z., Xie, X., Shen, R., Tan, J., Li, H., *et al*. (2017) Development of "purple endosperm rice" by engineering anthocyanin biosynthesis in the endosperm with a high-efficiency transgene stacking system. Mol. Plant, 10, 918−929
65. Lin, L., Liu, Y. G., Xu, X. and Li, B. (2003) Efficient linking and transfer of multiple genes by a multigene assembly and transformation vector system. Proc. Natl. Acad. Sci. USA, 100, 5962−5967
66. Zhao, Y., Han, J., Tan, J., Yang, Y., Li, S., Gou, Y., Luo, Y., Li, T., Xiao, W., Xue, Y., *et al*. (2022) Efficient assembly of long DNA fragments and multiple genes with improved nickase-based cloning and Cre/loxP recombination. Plant Biotechnol. J., 20, 1983−1995
67. Altpeter, F., Springer, N. M., Bartley, L. E., Blechl, A. E., Brutnell, T. P., Citovsky, V., Conrad, L. J., Gelvin, S. B., Jackson, D. P., Kausch, A. P., *et al*. (2016) Advancing crop transformation in the era of genome editing. Plant Cell, 28, 1510−1520
68. Schaumberg, K. A., Antunes, M. S., Kassaw, T. K., Xu, W., Zalewski, C. S., Medford, J. I. and Prasad, A. (2016) Quantitative characterization of genetic parts and circuits for plant synthetic biology. Nat. Methods, 13, 94−100
69. Matsuo, N., Minami, M., Maeda, T. and Hiratsuka, K. (2001) Dual luciferase assay for monitoring transient gene expression in higher plants. Plant Biotechnol. (Tsukuba), 18, 71−75
70. 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, 32, 2120−2131
71. Kim, Y. S., Johnson, G. D., Seo, J., Barrera, A., Cowart, T. N., Majoros, W. H., Ochoa, A., Allen, A. S. and Reddy, T. E. (2021) Correcting signal biases and detecting regulatory elements in STARR-seq data. Genome Res., 31, 877−889
72. Jores, T., Tonnies, J., Wrightsman, T., Buckler, E. S., Cuperus, J. T., Fields, S. and Queitsch, C. (2021) Synthetic promoter designs enabled by a comprehensive analysis of plant core promoters. Nat. Plants, 7, 842−855
73. 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. Genom. Proteom. Bioinf., 17, 140−153
74. Tian, C., Zhang, Y., Li, J. and Wang, Y. (2022) Benchmarking intrinsic promoters and terminators for plant synthetic biology research. BioDesign Research., 9834989
75. Andreou, A. I., Nirkko, J., Ochoa-Villarreal, M. and Nakayama, N. (2021) Mobius assembly for plant systems highlights promoter-terminator interaction in gene regulation. bioRxiv doi: 10.1101/2021.03.31.437819
76. Gunadi, A., Rushton, P. J., Mchale, L. K., Gutek, A. H. and Finer, J. J. (2016) Characterization of 40 soybean (*Glycine max*) promoters, isolated from across 5 thematic gene groups. Plant Cell Tissue Organ Cult., 127, 1−16
77. Kakei, Y., Masuda, H., Nishizawa, N. K., Hattori, H. and Aung, M. S. (2021) Elucidation of novel cis-regulatory elements and promoter structures involved in iron excess response mechanisms in rice using a bioinformatics approach. Front. Plant Sci., 12, 660303
78. Kaur, A., Pati, P. K., Pati, A. M. and Nagpal, A. K. (2017) *In-silico* analysis of cis-acting regulatory elements of pathogenesis-related proteins of *Arabidopsis thaliana* and *Oryza sativa*. PLoS One, 12, e0184523
79. Schmitz, R. J., Grotewold, E. and Stam, M. (2022) Cis-regulatory sequences in plants: their importance, discovery, and future challenges. Plant Cell, 34, 718−741
80. Basu, D. and South, P. F. (2022) Design and analysis of native photorespiration gene motifs of promoter untranslated region combinations under short term abiotic stress conditions. Front. Plant Sci., 13, 828729
81. To, J. P. C., Davis, I. W., Marengo, M. S., Shariff, A., Baublite, C., Decker, K., Galvão, R. M., Gao, Z., Haragutchi, O., Jung, J. W., *et al*. (2021) Expression elements derived from plant sequences provide effective gene expression regulation and new opportunities for plant biotechnology traits. Front. Plant Sci., 12, 712179
82. Davis, I. W., Benninger, C., Benfey, P. N. and Elich, T. (2012) POWRS: position-sensitive motif discovery. PLoS One, 7, e40373
83. McCarthy, D. M. and Medford, J. I. (2020) Quantitative and predictive genetic parts for plant synthetic biology. Front. Plant Sci., 11, 512526
84. Han, L., Silvestre, S., Sayanova, O., Haslam, R. P. and Napier, J. A. (2022) Using field evaluation and systematic iteration to rationalise the accumulation of omega-3 long-chain polyunsaturated fatty acids in transgenic *Camelina sativa*. Plant Biotechnol. J., 20, 1833−1852
85. Petrie, J. R., Zhou, X. R., Leonforte, A., McAllister, J., Shrestha, P., Kennedy, Y., Belide, S., Buzza, G., Gororo, N., Gao, W., *et al*. (2020) Development of a *Brassica napus* (Canola) crop containing fish oil-like levels of DHA in the seed oil. Front. Plant Sci., 11, 727
86. Belide, S., Shrestha, P., Kennedy, Y., Leonforte, A., Devine, M. D., Petrie, J. R., Singh, S. P. and Zhou, X. R. (2022) Engineering docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in *Brassica juncea*. Plant Biotechnol. J., 20, 19−21
87. Yan-yan, L., Li-na, G., Cheng-zhen, L. and Zhi-gang, M. Li, Y., Guo, L., Liang, C., Meng, Z., Tahira, S., Guo, S. and Zhang, R. (2022) Overexpression of *Brassica napus* cytosolic fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase genes significantly enhanced tobacco growth and biomass. J. Integr. Agric., 21, 49−59
88. Fan, H., Liu, Y., Li, C. Y., Jiang, Y., Song, J. J., Yang, L., Zhao, Q., Hu, Y. H., Chen, X. Y. and Xu, J. J. (2021) Engineering high coenzyme Q_{10} tomato. Metab. Eng., 68, 86−93
89. Forestier, E. C. F., Czechowski, T., Cording, A. C., Gilday, A. D., King, A. J., Brown, G. D. and Graham, I. A. (2021) Developing a *Nicotiana benthamiana* transgenic platform for high-value diterpene production and candidate gene evaluation. Plant Biotechnol. J., 19, 1614−1623

© The Author(s) 2023. Published by Higher Education Press

90. Davis, K., Gkotsi, D. S., Smith, D. R. M., Goss, R. J. M., Caputi, L. and O'Connor, S. E. (2020) *Nicotiana benthamiana* as a transient expression host to produce auxin analogs. Front. Plant Sci., 11, 581675
91. Allen, Q. M., Febres, V. J., Rathinasabapathi, B. and Chaparro, J. X. (2022) Engineering a plant-derived astaxanthin synthetic pathway into *Nicotiana benthamiana*. Front. Plant Sci., 12, 831785
92. Narayanan, N., Beyene, G., Chauhan, R. D., Gaitán-Solís, E., Gehan, J., Butts, P., Siritunga, D., Okwuonu, I., Woll, A., Jiménez-Aguilar, D. M., *et al*. (2019) Biofortification of field-grown cassava by engineering expression of an iron transporter and ferritin. Nat. Biotechnol., 37, 144−151
93. Liang, Q., Wang, K., Liu, X., Riaz, B., Jiang, L., Wan, X., Ye, X. and Zhang, C. (2019) Improved folate accumulation in genetically modified maize and wheat. J. Exp. Bot., 70, 1539−1551
94. Liu, X., Ma, X., Wang, H., Li, S., Yang, W., Nugroho, R. D., Luo, L., Zhou, X., Tang, C., Fan, Y., *et al*. (2021) Metabolic engineering of astaxanthin-rich maize and its use in the production of biofortified eggs. Plant Biotechnol. J., 19, 1812−1823
95. Nett, R. S., Lau, W. and Sattely, E. S. (2020) Discovery and engineering of colchicine alkaloid biosynthesis. Nature, 584, 148−153
96. Li, J., Mutanda, I., Wang, K., Yang, L., Wang, J. and Wang, Y. (2019) Chloroplastic metabolic engineering coupled with isoprenoid pool enhancement for committed taxanes biosynthesis in *Nicotiana benthamiana*. Nat. Commun., 10, 4850
97. Mateos-Fernández, R., Moreno-Giménez, E., Gianoglio, S., Quijano-Rubio, A., Gavaldá-García, J., Estellés, L., Rubert, A., Rambla, J. L., Vazquez-Vilar, M., Huet, E., *et al*. (2021) Production of volatile moth sex pheromones in transgenic *Nicotiana benthamiana* plants. BioDesign Research., 2021, 9891082
98. Iacopino, S., Jurinovich, S., Cupellini, L., Piccinini, L., Cardarelli, F., Perata, P., Mennucci, B., Giuntoli, B. and Licausi, F. (2019) A synthetic oxygen sensor for plants based on animal hypoxia signaling. Plant Physiol., 179, 986−1000
99. Nemhauser, J. L. and Torii, K. U. (2016) Plant synthetic biology for molecular engineering of signalling and development. Nat. Plants, 2, 16010
100. Swinnen, G., Goossens, A. and Pauwels, L. (2016) Lessons from domestication: targeting cis-regulatory elements for crop improvement. Trends Plant Sci., 21, 506−515
101. Andres, J., Blomeier, T. and Zurbriggen, M. D. (2019) Synthetic switches and regulatory circuits in plants. Plant Physiol., 179, 862−884
102. Zhou, Y., Ding, M., Gao, S., Yu-Strzelczyk, J., Krischke, M., Duan, X., Leide, J., Riederer, M., Mueller, M. J., Hedrich, R., *et al*. (2021) Optogenetic control of plant growth by a microbial rhodopsin. Nat. Plants, 7, 144−151
103. Liu, L., Gallagher, J., Arevalo, E. D., Chen, R., Skopelitis, T., Wu, Q., Bartlett, M. and Jackson, D. (2021) Enhancing grain-yield-related traits by CRISPR-Cas9 promoter editing of maize *CLE* genes. Nat. Plants, 7, 287−294
104. Rodríguez-Leal, D., Lemmon, Z. H., Man, J., Bartlett, M. E. and Lippman, Z. B. (2017) Engineering quantitative trait variation for crop improvement by genome editing. Cell, 171, 470−480.e8
105. Song, X., Meng, X., Guo, H., Cheng, Q., Jing, Y., Chen, M., Liu, G., Wang, B., Wang, Y., Li, J., *et al*. (2022) Targeting a gene regulatory element enhances rice grain yield by decoupling panicle number and size. Nat. Biotechnol., 40, 1403−1411
106. Lowder, L. G., Zhou, J., Zhang, Y., Malzahn, A., Zhong, Z., Hsieh, T. F., Voytas, D. F., Zhang, Y. and Qi, Y. (2018) Robust transcriptional activation in plants using multiplexed CRISPR-Act2. 0 and mTALE-Act systems. Mol. Plant, 11, 245−256
107. Lowder, L. G., Paul, J. W. 3rd and Qi, Y. (2017) Multiplexed transcriptional activation or repression in plants using CRISPR-dCas9-based systems. Methods Mol. Biol., 1629, 167−184
108. Lowder, L. G., Zhang, D., Baltes, N. J., Paul, J. W. 3rd, Tang, X., Zheng, X., Voytas, D. F., Hsieh, T. F., Zhang, Y. and Qi, Y. (2015) A CRISPR/Cas9 toolbox for multiplexed plant genome editing and transcriptional regulation. Plant Physiol., 169, 971−985
109. Chavez, A., Scheiman, J., Vora, S., Pruitt, B. W., Tuttle, M., P R Iyer, E., Lin, S., Kiani, S., Guzman, C. D., Wiegand, D. J., *et al*. (2015) Highly efficient Cas9-mediated transcriptional programming. Nat. Methods, 12, 326−328
110. Sajwan, S. and Mannervik, M. (2019) Gene activation by dCas9-CBP and the SAM system differ in target preference. Sci. Rep., 9, 18104
111. Zalatan, J. G., Lee, M. E., Almeida, R., Gilbert, L. A., Whitehead, E. H., La Russa, M., Tsai, J. C., Weissman, J. S., Dueber, J. E., Qi, L. S., *et al*. (2015) Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds. Cell, 160, 339−350
112. Zhou, H., Liu, J., Zhou, C., Gao, N., Rao, Z., Li, H., Hu, X., Li, C., Yao, X., Shen, X., *et al*. (2018) *In vivo* simultaneous transcriptional activation of multiple genes in the brain using CRISPR-dCas9-activator transgenic mice. Nat. Neurosci., 21, 440−446
113. Li, Z., Zhang, D., Xiong, X., Yan, B., Xie, W., Sheen, J. and Li, J. F. (2017) A potent Cas9-derived gene activator for plant and mammalian cells. Nat. Plants, 3, 930−936
114. Pan, C., Wu, X., Markel, K., Malzahn, A. A., Kundagrami, N., Sretenovic, S., Zhang, Y., Cheng, Y., Shih, P. M. and Qi, Y. (2021) CRISPR-Act3. 0 for highly efficient multiplexed gene activation in plants. Nat. Plants, 7, 942−953
115. Selma, S., Bernabé-Orts, J. M., Vazquez-Vilar, M., Diego-Martin, B., Ajenjo, M., Garcia-Carpintero, V., Granell, A. and Orzaez, D. (2019) Strong gene activation in plants with genome-wide specificity using a new orthogonal CRISPR/Cas9-based programmable transcriptional activator. Plant Biotechnol. J., 17, 1703−1705
116. Selma, S., Sanmartín, N., Espinosa-Ruiz, A., Gianoglio, S., Lopez-Gresa, M. P., Vázquez-Vilar, M., Flors, V., Granell, A.

 © The Author(s) 2023. Published by Higher Education Press

and Orzaez, D. (2022) Custom-made design of metabolite composition in *N.* benthamiana leaves using CRISPR activators. Plant Biotechnol. J., 20, 1578−1590
117. Pan, C., Li, G., Malzahn, A. A., Cheng, Y., Leyson, B., Sretenovic, S., Gurel, F., Coleman, G. D. and Qi, Y. (2022) Boosting plant genome editing with a versatile CRISPR-Combo system. Nat. Plants, 8, 513−525
118. Dey, N., Sarkar, S., Acharya, S. and Maiti, I. B. (2015) Synthetic promoters in planta. Planta, 242, 1077−1094
119. Aysha, J., Noman, M., Wang, F., Liu, W., Zhou, Y., Li, H. and Li, X. (2018) Synthetic promoters: designing the cis regulatory modules for controlled gene expression. Mol. Biotechnol., 60, 608−620
120. Pandiarajan, R. and Grover, A. (2018) *In vivo* promoter engineering in plants: are we ready? Plant Sci., 277, 132–138
121. Ouma, W. Z., Pogacar, K. and Grotewold, E. (2018) Topological and statistical analyses of gene regulatory networks reveal unifying yet quantitatively different emergent properties. PLOS Comput. Biol., 14, e1006098
122. Core, L. J., Martins, A. L., Danko, C. G., Waters, C. T., Siepel, A. and Lis, J. T. (2014) Analysis of nascent RNA identifies a unified architecture of initiation regions at mammalian promoters and enhancers. Nat. Genet., 46, 1311−1320
123. Ricci, W. A., Lu, Z., Ji, L., Marand, A. P., Ethridge, C. L., Murphy, N. G., Noshay, J. M., Galli, M., Mejía-Guerra, M. K., Colomé-Tatché, M., *et al*. (2019) Widespread long-range cis-regulatory elements in the maize genome. Nat. Plants, 5, 1237−1249
124. Fagny, M., Kuijjer, M. L., Stam, M., Joets, J., Turc, O., Rozière, J., Pateyron, S., Venon, A. and Vitte, C. (2021) Identification of key tissue-specific, biological processes by integrating enhancer information in maize gene regulatory networks. Front. Genet., 11, 606285
125. Ding, W., Cheng, J., Guo, D., Mao, L., Li, J., Lu, L., Zhang, Y., Yang, J. and Jiang, H. (2018) Engineering the 5′UTR-mediated regulation of protein abundance in yeast using nucleotide sequence activity relationships. ACS Synth. Biol., 7, 2709−2714
126. Mutalik, V. K., Guimaraes, J. C., Cambray, G., Lam, C., Christoffersen, M. J., Mai, Q. A., Tran, A. B., Paull, M., Keasling, J. D., Arkin, A. P., *et al*. (2013) Precise and reliable gene expression via standard transcription and translation initiation elements. Nat. Methods, 10, 354−360
127. Chen, Y., Zhang, S., Young, E. M., Jones, T. S., Densmore, D. and Voigt, C. A. (2020) Genetic circuit design automation for yeast. Nat. Microbiol., 5, 1349−1360
128. Xia, P. F., Ling, H., Foo, J. L. and Chang, M. W. (2019) Synthetic genetic circuits for programmable biological functionalities. Biotechnol. Adv., 37, 107393
129. Van Brempt, M., Clauwaert, J., Mey, F., Stock, M., Maertens, J., Waegeman, W. and De Mey, M. (2020) Predictive design of sigma factor-specific promoters. Nat. Commun., 11, 5822
130. Nielsen, A. A., Der, B. S., Shin, J., Vaidyanathan, P., Paralanov, V., Strychalski, E. A., Ross, D., Densmore, D. and Voigt, C. A. (2016) Genetic circuit design automation. Science, 352, aac7341
131. Jones, T. S., Oliveira, S. M. D., Myers, C. J., Voigt, C. A. and Densmore, D. (2022) Genetic circuit design automation with Cello 2. 0. Nat. Protoc., 17, 1097−1113
132. Kwok, R. (2010) Five hard truths for synthetic biology. Nature, 463, 288−290
133. Shin, J., Zhang, S., Der, B. S., Nielsen, A. A. and Voigt, C. A. (2020) Programming *Escherichia coli* to function as a digital display. Mol. Syst. Biol., 16, e9401
134. Crowther, M., Wipat, A. and Goñi-Moreno, Á. (2022) A network approach to genetic circuit designs. ACS Synth. Biol., 11, 3058−3066
135. Pérez-González, A. and Caro, E. (2019) Benefits of using genomic insulators flanking transgenes to increase expression and avoid positional effects. Sci. Rep., 9, 8474
136. Puchta, H., Jiang, J., Wang, K. and Zhao, Y. (2022) Updates on gene editing and its applications. Plant Physiol., 188, 1725−1730
137. O’Neill, B. M., Mikkelson, K. L., Gutierrez, N. M., Cunningham, J. L., Wolff, K. L., Szyjka, S. J., Yohn, C. B., Redding, K. E. and Mendez, M. J. (2012) An exogenous chloroplast genome for complex sequence manipulation in algae. Nucleic Acids Res., 40, 2782−2792
138. Shao, Y., Lu, N., Wu, Z., Cai, C., Wang, S., Zhang, L. L., Zhou, F., Xiao, S., Liu, L., Zeng, X., *et al*. (2018) Creating a functional single-chromosome yeast. Nature, 560, 331−335
139. Tomita, M. (2001) Whole-cell simulation: a grand challenge of the 21st century. Trends Biotechnol., 19, 205−210
140. Karr, J. R., Sanghvi, J. C., Macklin, D. N., Gutschow, M. V., Jacobs, J. M., Bolival, B. Jr, Assad-Garcia, N., Glass, J. I. and Covert, M. W. (2012) A whole-cell computational model predicts phenotype from genotype. Cell, 150, 389−401
141. Macklin, D. N., Ahn-Horst, T. A., Choi, H., Ruggero, N. A., Carrera, J., Mason, J. C., Sun, G., Agmon, E., DeFelice, M. M., Maayan, I., *et al*. (2020) Simultaneous cross-evaluation of heterogeneous *E.* coli datasets via mechanistic simulation. Science, 369, eaav3751
142. Maritan, M., Autin, L., Karr, J., Covert, M. W., Olson, A. J. and Goodsell, D. S. (2022) Building structural models of a whole mycoplasma cell. J. Mol. Biol., 434, 167351
143. Lu, H., Kerkhoven, E. J. and Nielsen, J. (2022) Multiscale models quantifying yeast physiology: towards a whole-cell model. Trends Biotechnol., 40, 291−305
144. Beard, D. A., Neal, M. L., Tabesh-Saleki, N., Thompson, C. T., Bassingthwaighte, J. B., Shimoyama, M. and Carlson, B. E. (2012) Multiscale modeling and data integration in the virtual physiological rat project. Ann. Biomed. Eng., 40, 2365−2378
145. Marshall-Colon, A., Long, S. P., Allen, D. K., Allen, G., Beard, D. A., Benes, B., von Caemmerer, S., Christensen, A. J., Cox, D. J., Hart, J. C., *et al*. (2017) Crops *in silico*: generating virtual crops using an integrative and multi-scale modeling platform. Front. Plant Sci., 8, 786
146. Zhu, X. G., Lynch, J. P., LeBauer, D. S., Millar, A. J., Stitt, M. and Long, S. P. (2016) Plants *in silico*: why, why now and what?—an integrative platform for plant systems biology

research Plant Cell Environ., 39, 1049−1057

147. Zheng, H., Ho, P. Y., Jiang, M., Tang, B., Liu, W., Li, D., Yu, X., Kleckner, N. E., Amir, A. and Liu, C. (2016) Interrogating the *Escherichia coli* cell cycle by cell dimension perturbations. Proc. Natl. Acad. Sci. USA, 113, 15000−15005
148. Du, P., Zhao, H., Zhang, H., Wang, R., Huang, J., Tian, Y., Luo, X., Luo, X., Wang, M., Xiang, Y., *et al*. (2020) *De novo* design of an intercellular signaling toolbox for multi-channel cell-cell communication and biological computation. Nat. Commun., 11, 4226
149. Milias-Argeitis, A., Rullan, M., Aoki, S. K., Buchmann, P. and Khammash, M. (2016) Automated optogenetic feedback control for precise and robust regulation of gene expression and cell growth. Nat. Commun., 7, 12546
150. Olson, E. J., Hartsough, L. A., Landry, B. P., Shroff, R. and Tabor, J. J. (2014) Characterizing bacterial gene circuit dynamics with optically programmed gene expression signals. Nat. Methods, 11, 449−455
151. Carignano, A., Chen, D. H., Mallory, C., Wright, R. C., Seelig, G. and Klavins, E. (2022) Modular, robust, and extendible multicellular circuit design in yeast. eLife, 11, e74540
152. Dorrity, M. W., Alexandre, C. M., Hamm, M. O., Vigil, A. L., Fields, S., Queitsch, C. and Cuperus, J. T. (2021) The regulatory landscape of *Arabidopsis thaliana* roots at single-cell resolution. Nat. Commun., 12, 3334
153. Farmer, A., Thibivilliers, S., Ryu, K. H., Schiefelbein, J. and Libault, M. (2021) Single-nucleus RNA and ATAC sequencing reveals the impact of chromatin accessibility on gene expression in *Arabidopsis* roots at the single-cell level. Mol. Plant, 14, 372−383
154. Okubo-Kurihara, E., Ali, A., Hiramoto, M., Kurihara, Y., Abouleila, Y., Abdelazem, E. M., Kawai, T., Makita, Y., Kawashima, M., Esaki, T., *et al*. (2022) Tracking metabolites at single-cell resolution reveals metabolic dynamics during plant mitosis. Plant Physiol., 189, 459−464
155. Marand, A. P., Chen, Z., Gallavotti, A. and Schmitz, R. J. (2021) A cis-regulatory atlas in maize at single-cell resolution. Cell, 184, 3041−3055.e21

© The Author(s) 2023. Published by Higher Education Press