

Synthetic genetic circuits enabled precise reprogramming for developing high lysine maize

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Amino acids that are not *de novo* produced in the human body are known as essential amino acids. Among them, Lysine (Lys) is vital, which is limited to most of the cereal grains like maize [1]. Maize has a significant contribution to global food security. Although several biotechnological approaches have shown a successful increase of maize Lys, a comprehensive strategy to increase maize Lys following synthetic genetic circuit enabled reprogramming still lacks and needs to be developed. Synthetic genetic circuit technology has been challenging to implement in plant/maize as transgenesis needs a long time, and tuning circuit activity in heterogenous cells is difficult [2]. Quantitative transient expression assays could be done to construct synthetic maize for testing and tuning circuit designs. To construct synthetic genetic circuits in maize, synthetic transcriptional regulators need to be generated. Synthetic promoters, transcriptional activators, and repressors need to be designed and the system should be optimized [3]. The designed synthetic parts should function independently. In synthetic maize, external control of gene expression in the Lys biosynthesis pathway or other pathways that interact with Lys biosynthesis is needed, which transmits the input for genetic circuits.

For predicting the consistent function of engineered genetic circuits for Lys overproduction, analyzing and quantitatively assessing the function of fabricated components are crucial. Hence, complex genetic circuits should incorporate predictable, electronic-like functionalities such as negative/positive feedback loops, switches, and Boolean logic gates [4]. In addition, system biology-mediated synthesis of autoregulatory transcriptional feedback loops (negative or positive) could be similarly done [5].

In these synthetic circuits for over producing of Lys, the transcription factor regulates its own expression, either positive or negative control mechanism, operating especially at the transcriptional level. Additionally, a synthetic positive feedback loop in the high Lys genetic circuit could significantly minimize the time needed to build the system at a steady state [6].

When activated, positive feedback loops amplify weak signals and facilitate cellular memory to the input signal. It is employed to design a positive feedback loop in the protoplasts of *Arabidopsis* and lettuce [7]. Furthermore, negative feedback circuits minimize the implications of perturbations, or noise, in the cell system [8]. In natural systems, sensitivity and homeostasis are regulated by positive and negative feedback systems, respectively, which should be considered during

designing synthetic circuits [9].

Toggle switches were some of the earliest synthetic genetic circuits and were found in prokaryotic cells [9]. The half-maximum concentration of repressor, Hill coefficient, and promoter strength could be analyzed to produce a functional toggle switch. In the synthetic genetic circuit, the exogenous inducer switches between two stable states that directly affect the repressor activity [10]. Synthetic Boolean logic gates could allow maize to execute complicated cellular computations, for overproducing Lys. CRISPR-Cas9 BLADE might be effective for transcriptional reprogramming of the Lys biosynthesis process. Synthetic feedback loops in the Lys biosynthesis pathway might improve the scope of synthetic logic [5]. Such complex circuits in the Lys biosynthesis pathway could inspire synthetic biologists to develop other desirable characteristics.

Even though synthetic plant biology is still in its early stages, it provides a distinctive and potent platform for synthetic biology. The synthetic circuit for high Lys maize targeting the Lys biosynthesis pathway like an aspartate pathway or other pathways, transcription factors transporters should be considered during developing synthetic genetic circuits enabled precise reprogramming for high Lys maize (Figure 1).

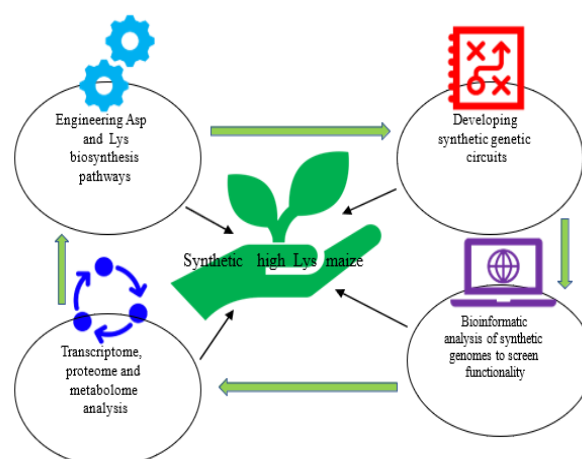


Figure 1. Systematic approaches for developing high Lys maize following synthetic genetic circuit enabled reprogramming of the maize genome.

Disclosure statement

No potential conflict of interest was reported by the author.

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