Synthetic DNA tools to speed up the construction and testing of genetic circuits

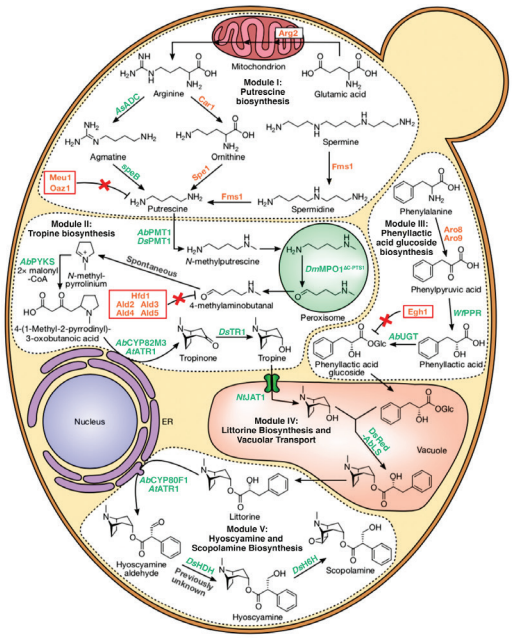
Synthetic genetic circuits are networks of genes that have been engineered to co-opt a cell's machinery and bring about an atypical, but useful function or trait. Building genetic circuitry often requires synthesis of epistatic networks of genes, regulatory sequences, and key effectors who's collective activity result in a desired outcome. Recent successful applications of genetic circuits have ranged from the production of plant-derived therapeutics on an industrial scale to the formation of low-cost point-of-care diagnostic reporters[1–4].

BACKGROUND/CHALLENGES

Creating genetic circuitry often follows the iterative cycle of design, build, test and learn. A bottleneck in this workflow comes when building and testing the myriad combinations of pathway parts that impact a pathway's optimal function.

Twist Bioscience's silicon-based DNA synthesis platform enables large-scale and highly precise synthesis of genetic components. We build your DNA constructs at the scale you need, helping you test and learn more efficiently.

Researchers like Srinivasan and Smolke (2020) have used Twist Gene Synthesis to multiplex the design-build-test-learn cycle and generate complex genetic circuits with iteration cycles as short as 3 weeks[3,5].



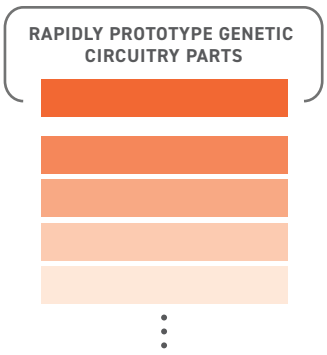
Example of genetic circuit created for the modular production of the plant-derived scopolamine in yeast[3,5]. Modules are outlined. Color scheme: orange - overexpressed yeast genes; green - heterologous genes synthesized as Gene Fragments. Red boxes - disrupted yeast genes

Tools to Support the Efficient Building, Testing, and Optimization of Genetic Circuitry

SYNTHETIC GENES

The efficient engineering of genetic circuitry requires the rapid testing of different genes and gene pathway combinations. Twist can rapidly synthesize your pathway components, and assemble them into your vector, ready for testing out-of-the-box.

- **Rapidly prototype.** Double-stranded genes arrive in as little as 6 business days. Cloned genes arrive in as little as 11 business days.
- **Avoid building, focus on testing.** Send us your vector, and we will handle the cloning so you can focus on the science that matters.
- **Synthesize entire pathways.** Gene synthesis is available up to 5 kb, meaning entire pathways can be encoded in a single sequence. Need longer than 5 kb? Contact us using the details on the next page.
- **DNA at scales to suit your experiment.** Some applications necessitate more than a miniprep of DNA. Cloned genes are available up to 1 mg scales.

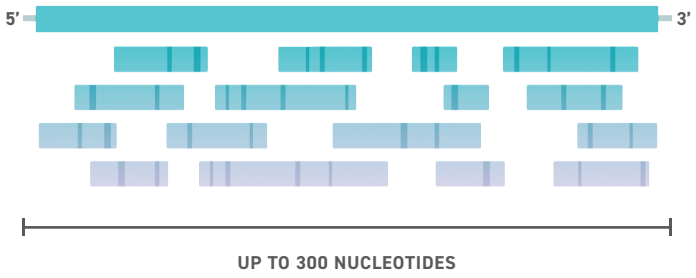


Twist's gene synthesis process allows for easy generation of genetic components, enabling accurate production and rapid testing of different circuitry combinations.

OLIGO POOLS

Twist's multiplexed DNA synthesis platform enables precise assembly of oligonucleotides up to 300 nucleotides in length. With the ability to produce over a million unique oligos in a single run, Twist Oligo Pools facilitate parallel building and testing of short sequences during genetic circuit optimization.

- **Encode genetic part libraries in Oligo Pools.** 300 nt includes the majority of known effectors, promoters, terminators and signaling peptides.
- **Focus on everything that matters.** Oligo Pool sizes are fully customizable. Synthesize hundreds to millions of pathway parts at once.
- **No part left behind.** Highly uniform oligo synthesis ensures minimal oversampling in high throughput screens.
- **Skip amplification and cloning.** Oligo-encoded libraries of parts can be delivered already cloned into a vector of your choice.



With oligo lengths up to 300nt it's possible to encode multiple elements in a single design giving you the ability to discover the ideal part for your genetic circuit.

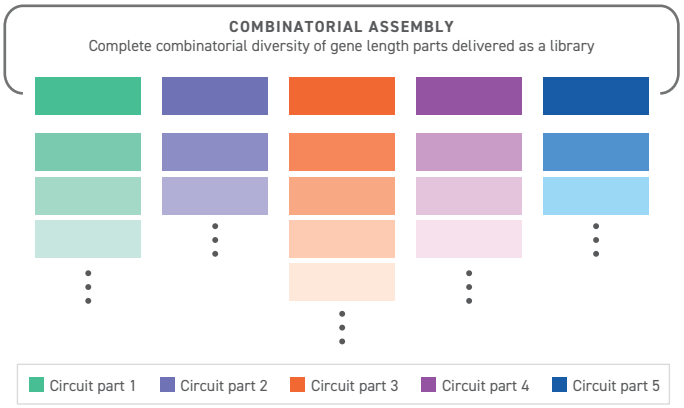
COMBINATORIAL ASSEMBLY OF PATHWAY PARTS

EARLY ACCESS

Identifying the set of genetic parts that leads to an optimal output is a significant and multidimensional challenge.

Twist Bioscience's combinatorial assembly service generates a library of pathways that can be rapidly tested.

Define the sets of gene-length parts that make up your pathway, and Twist will generate a library containing every possible combination of genes. The libraries arrive cloned, so you can achieve comprehensive, highly parallelized testing of your genetic circuitry.



Twist Combinatorial Variant Libraries can be used to design a library of pathway variants containing multiple genes in variable combinations.

TALK TO US ABOUT YOUR NEXT GENETIC CIRCUITS PROJECT.

Contact the Twist Bioscience team at **support@twistbioscience.com** or visit **twistbioscience.com**

REFERENCES

Brophy, Jennifer A N, and Christopher A Voigt. "Principles of Genetic Circuit Design." Nature Methods, vol. 11, no. 5, 29 Apr. 2014, pp. 508–520, 10.1038/nmeth.2926. Accessed 16 Feb. 2020.

Mansouri, Maysam, and Martin Fussenegger. "Therapeutic Cell Engineering: Designing Programmable Synthetic Genetic Circuits in Mammalian Cells." Protein & Cell, 29 Sept. 2021, 10.1007/s13238-021-00876-1. Accessed 19 Nov. 2021.

Amalfitano, Evan, et al. "A Glucose Meter Interface for Point-of-Care Gene Circuit-Based Diagnostics." Nature Communications, vol. 12, no. 1, 1 Feb. 2021, p. 724, www.nature.com/articles/s41467-020-20639-6, 10.1038/s41467-020-20639-6. Accessed 4 Mar. 2021.

"Rapid, Whole-Cell Engineering of Plant Alkaloid Biosynthesis in Yeast Using Twist Gene Fragments | Twist Bioscience." www.twistbioscience.com, www.twistbioscience.com/resources/application-note/rapid-whole-cell-engineering-plant-alkaloid-biosynthesis-yeast-using. Accessed 18 Mar. 2022.

Srinivasan, Prashanth, and Christina D. Smolke. "Biosynthesis of Medicinal Tropane Alkaloids in Yeast." Nature, vol. 585, no. 7826, 1 Sept. 2020, pp. 614–619, www.nature.com/articles/s41586-020-2650-9, 10.1038/s41586-020-2650-9.

Kwok, Roberta. "Five Hard Truths for Synthetic Biology." Nature, vol. 463, no. 7279, Jan. 2010, pp. 288–290, 10.1038/463288a. Accessed 21 Apr. 2021.