GenScript
Make Research Easy

Building DNA Libraries to Explore the Combinatorial Design Space

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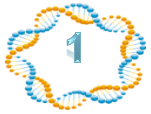
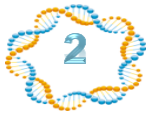
Outline



Combinatorial Optimization for Metabolic Pathway Engineering

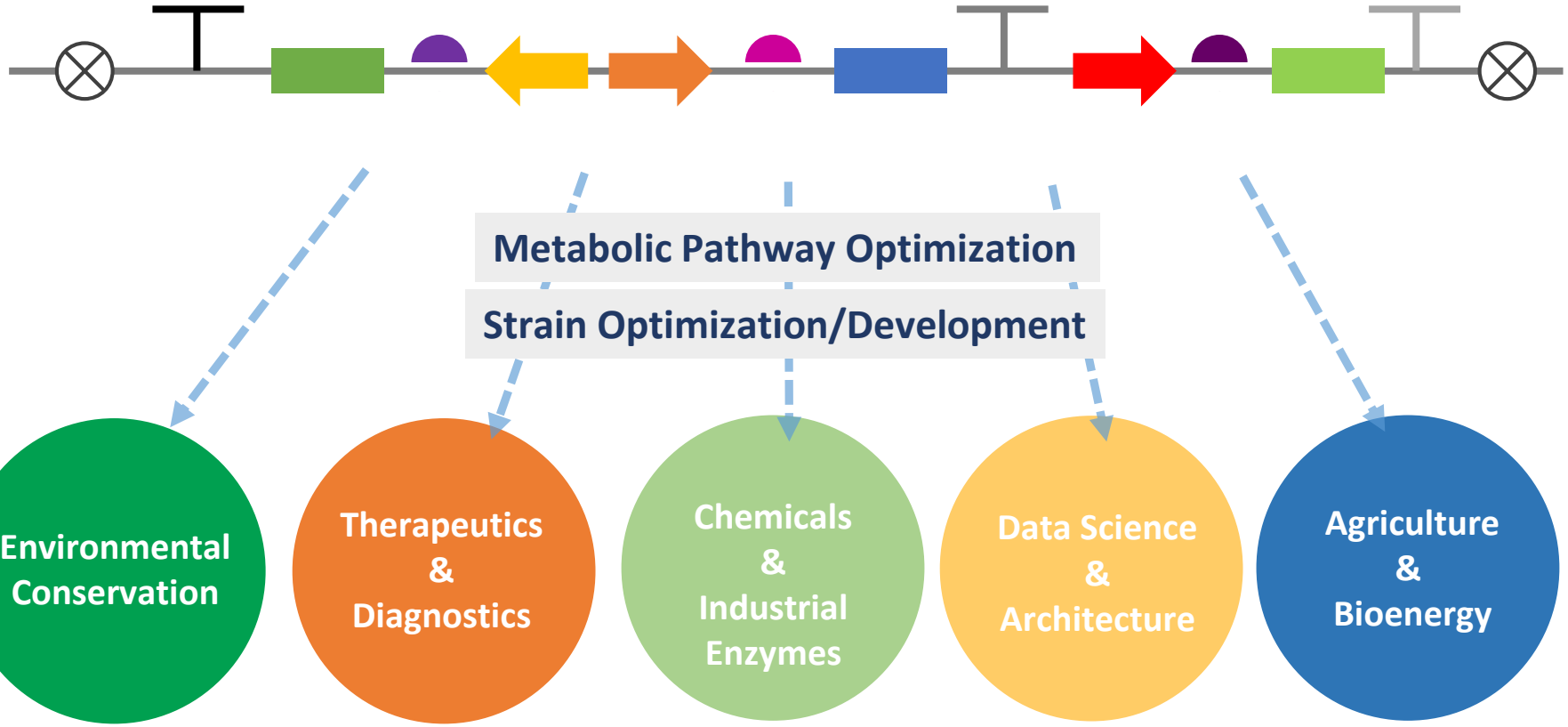


Case Studies for Combinatorial Optimization

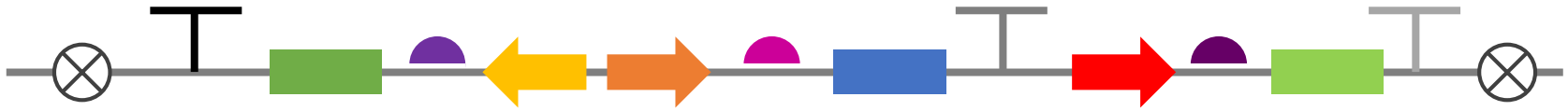
Combinatorial DNA Library Services

Building Metabolic Pathways and Gene Circuits for Diverse Applications

Using biological data and engineering principles to design and build novel biological systems or optimize natural systems in a more predictable and reliable manner for useful purposes.



Challenges in Engineering Biological Systems



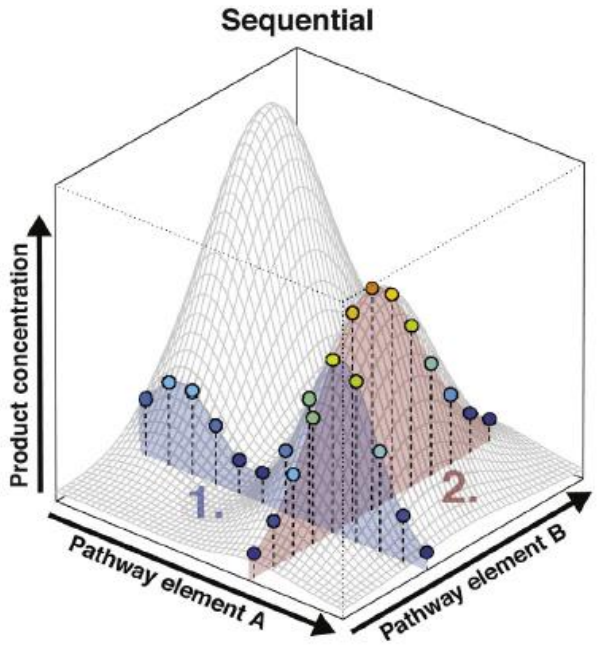
Lack of *a priori* knowledge of the system

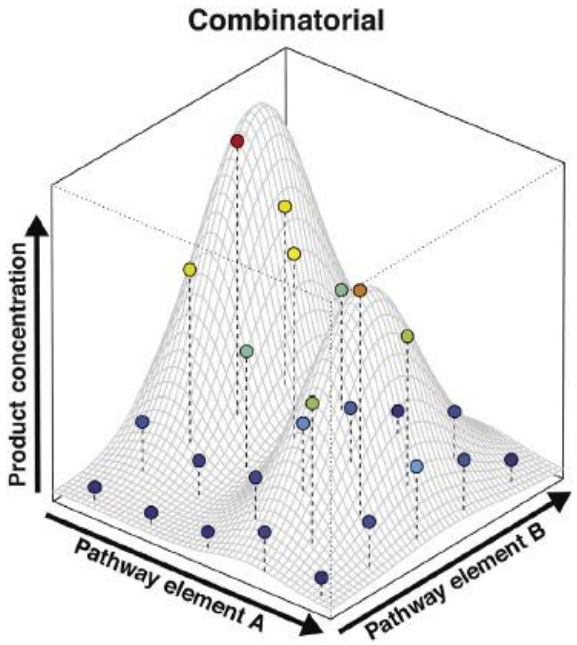
Intricate interactions involved in cellular metabolism

Lack of predictive models for the system

Not well-characterized parts for building the system

Sequential vs Combinatorial Optimization



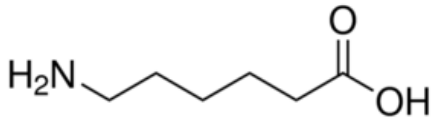


- For sequential pathway optimization, a pathway element is varied individually (blue curve) until a local optimum is identified.
- The best point is fixed as a starting point for the optimization of a second element (red curve) and so forth.

- For combinatorial pathway optimization, multiple elements are varied simultaneously.
- Combinatorial optimization allows for systematic screening of the multidimensional space and is more likely to reach a global optimum (red point).

Curr Opin Biotechnol. 2017 Oct;47:142-151.

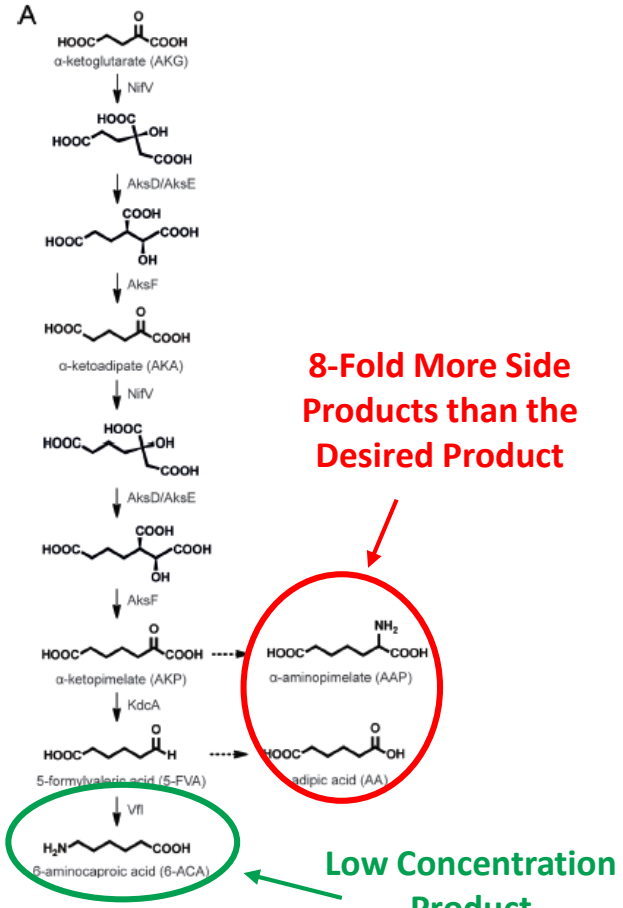
Biosynthetic Pathway for the Production of 6-ACA



6-aminocaproic acid (6-ACA)

Nylon

6-ACA Biosynthetic Pathway



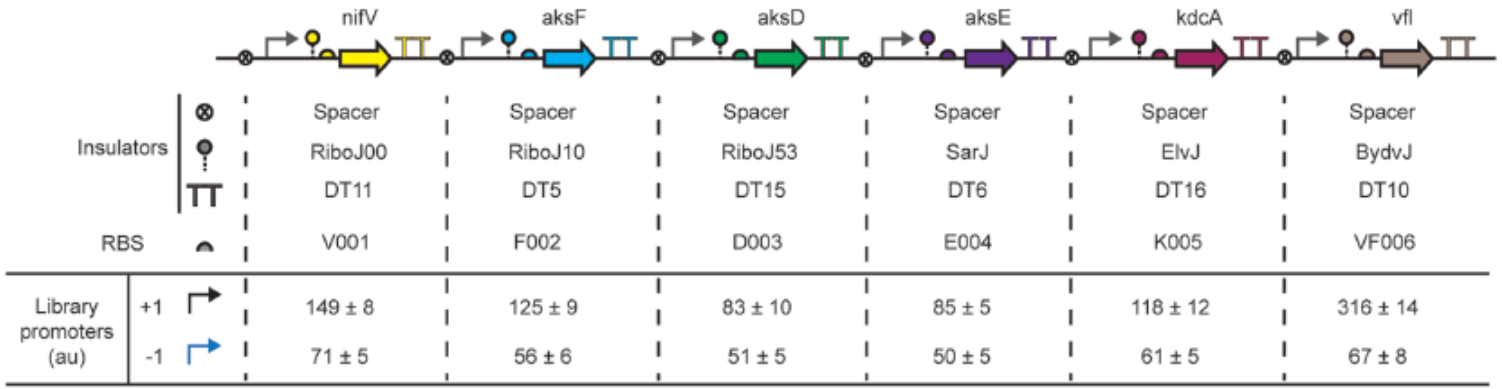
8-Fold More Side Products than the Desired Product

Low Concentration Product

- 6-ACA is the linear form of caprolactam, which is the chemical building block of nylon-6.
- The building block is currently produced from fossil-based chemical processes, which leads to significant greenhouse gas emission.
- The natural 6-ACA biosynthetic pathway consists of six heterologous enzymes.
- The existing cell factory:
  - produced the desired product at only [8 mg/L],
  - there was 8-fold more side products than the desired product in the broth.

Nucleic Acids Res. 2015 Dec 2;43(21):10560-70.

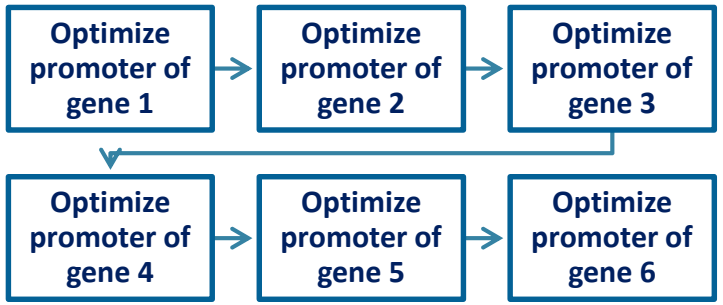
Pathway Optimization for 6-ACA Production



		nifV	aksF	aksD	aksE	kdcA	vfl
Insulators	⊗	Spacer	Spacer	Spacer	Spacer	Spacer	Spacer
		RiboJ00	RiboJ10	RiboJ53	SarJ	ElvJ	BydvJ
		DT11	DT5	DT15	DT6	DT16	DT10
RBS		V001	F002	D003	E004	K005	VF006
Library promoters (au)	+1	149 ± 8	125 ± 9	83 ± 10	85 ± 5	118 ± 12	316 ± 14
	-1	71 ± 5	56 ± 6	51 ± 5	50 ± 5	61 ± 5	67 ± 8

Testing strong vs weak expression for each of the six genes in the pathway

Sequential optimization



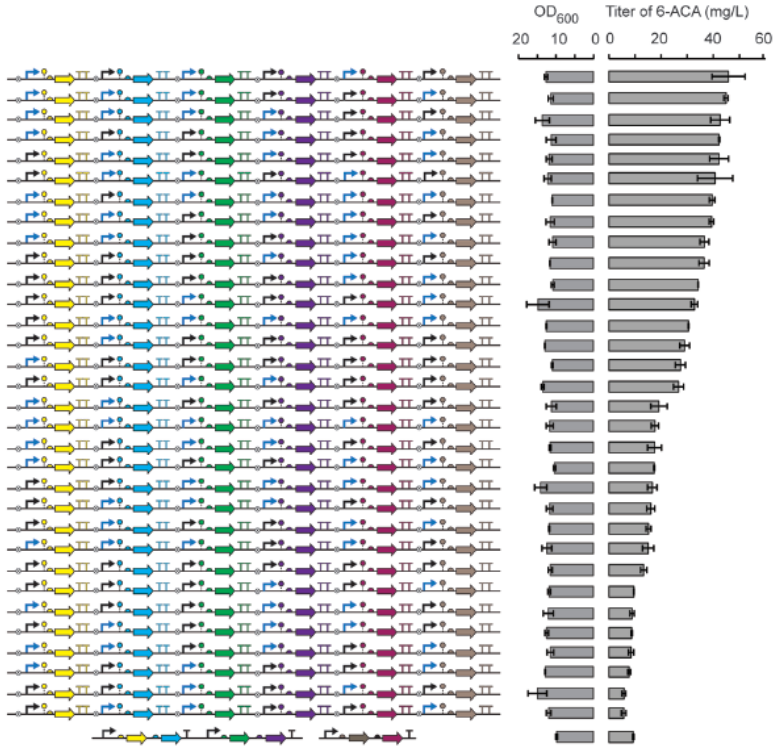
Combinatorial optimization

Optimizing promoters of six genes simultaneously

Nucleic Acids Res. 2015 Dec 2;43(21):10560-70.

Combinatorial Optimization for 6-ACA Production

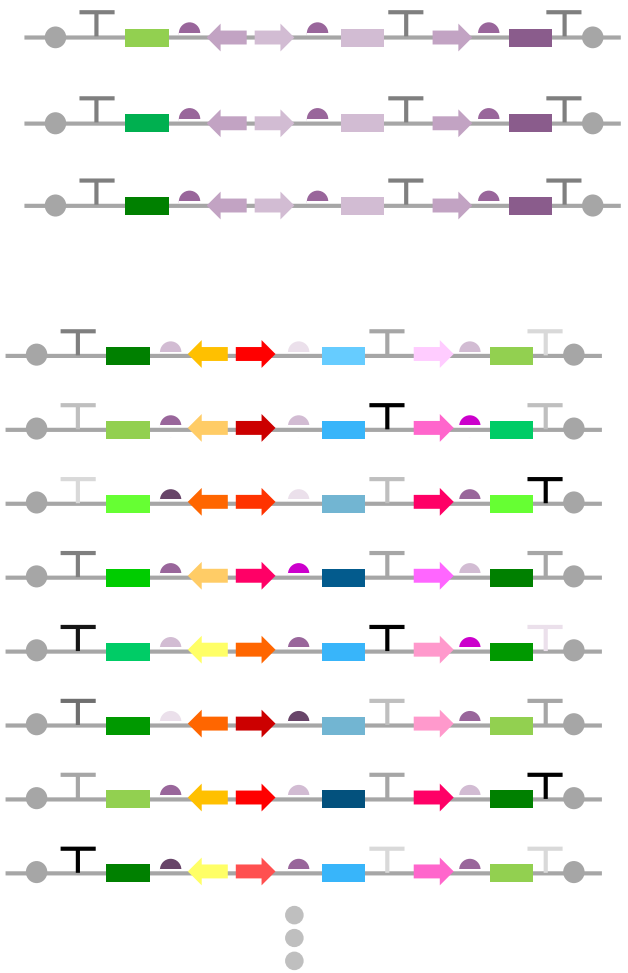
Cluster No.	Coding								
	nifV	aksF	aksD	aksE	kdcA	vfl	Fe^{3+}/C	Vit B_1	Mg^{2+}
20	-1	+1	-1	+1	-1	+1	-1	+1	+1
52	-1	-1	-1	+1	+1	-1	-1	+1	-1
14	-1	-1	-1	-1	+1	+1	+1	+1	+1
8	-1	+1	+1	-1	+1	-1	-1	+1	+1
9	+1	-1	+1	-1	+1	-1	-1	+1	-1
41	+1	-1	-1	+1	-1	+1	-1	+1	-1
56	-1	+1	-1	-1	-1	-1	+1	+1	-1
21	-1	-1	+1	-1	-1	+1	-1	+1	-1
12	-1	-1	+1	+1	-1	-1	+1	+1	+1
4	+1	+1	+1	-1	-1	+1	-1	+1	+1
25	+1	+1	+1	+1	-1	-1	+1	+1	-1
45	+1	+1	-1	+1	+1	-1	-1	+1	+1
39	+1	-1	-1	-1	-1	-1	+1	+1	+1
60	+1	-1	+1	+1	+1	+1	+1	+1	+1
11	-1	+1	+1	+1	+1	+1	+1	+1	-1
53	+1	+1	-1	-1	+1	+1	+1	+1	-1
32	-1	-1	+1	+1	+1	+1	-1	-1	+1
63	-1	-1	-1	-1	-1	-1	-1	-1	+1
51	-1	+1	-1	-1	+1	+1	-1	-1	-1
61	-1	+1	+1	+1	-1	-1	-1	-1	-1
24	+1	+1	-1	-1	-1	-1	-1	-1	-1
23	+1	-1	-1	-1	+1	+1	-1	-1	+1
54	+1	-1	+1	+1	-1	-1	-1	-1	+1
26	-1	-1	-1	+1	-1	+1	+1	-1	-1
1	+1	+1	+1	+1	+1	+1	-1	-1	-1
34	+1	+1	+1	-1	+1	-1	+1	-1	+1
48	-1	+1	+1	-1	-1	+1	+1	-1	+1
62	+1	-1	+1	-1	-1	+1	+1	-1	-1
17	-1	-1	+1	-1	+1	-1	+1	-1	-1
30	-1	+1	-1	+1	+1	-1	+1	-1	+1
35	+1	+1	-1	+1	-1	+1	+1	-1	+1
16	+1	-1	-1	+1	+1	-1	+1	-1	-1
eAKP672									



- Three additional factors to consider in media composition:
  - [ferric citrate]
  - [vitamin B1]
  - [magnesium ion (Mg2+)]
- In total, nine factors were optimized simultaneously.
- Total library size was 2^9, which equals to 512 different combinations.
- "Design of Experiment" was used to reduce combinatorial library size.
- Finally, with a single round of combinatorial optimization, 6-ACA production was increased by 5 folds.

Nucleic Acids Res. 2015 Dec 2;43(21):10560-70.

Combinatorial Optimization Requires a Powerful Assembly Method



Sequential optimization

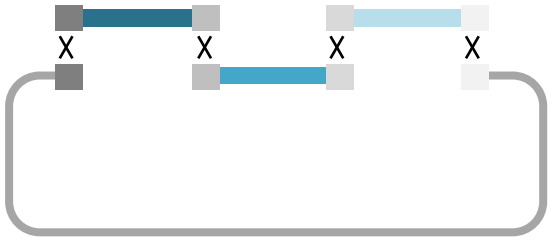
- Tests:
  - less than 10 constructs at a time
  - one part at a time
- Time-consuming and costly

Combinatorial optimization

- Tests:
  - hundreds and thousands constructs in parallel
  - multiple parts simultaneously
- Efficient and cost-effective

Pathway Assembly Platforms

In vitro Homologous Recombination

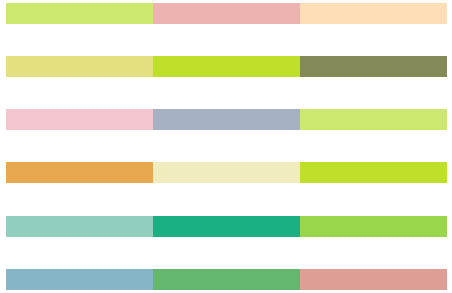


- Low throughput
- Low efficiency with >5 parts
- Expensive

Golden Gate Assembly

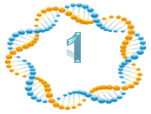
- Based on Type IIs enzyme
- Has sequence limitation

GenScript's Platform

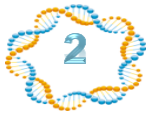


- High throughput
- No sequence limitation
- Seamless (no scars)
- Highly efficient with up to 10 parts and 20 kb insert size

Outline



Combinatorial Optimization for Metabolic Pathway Engineering

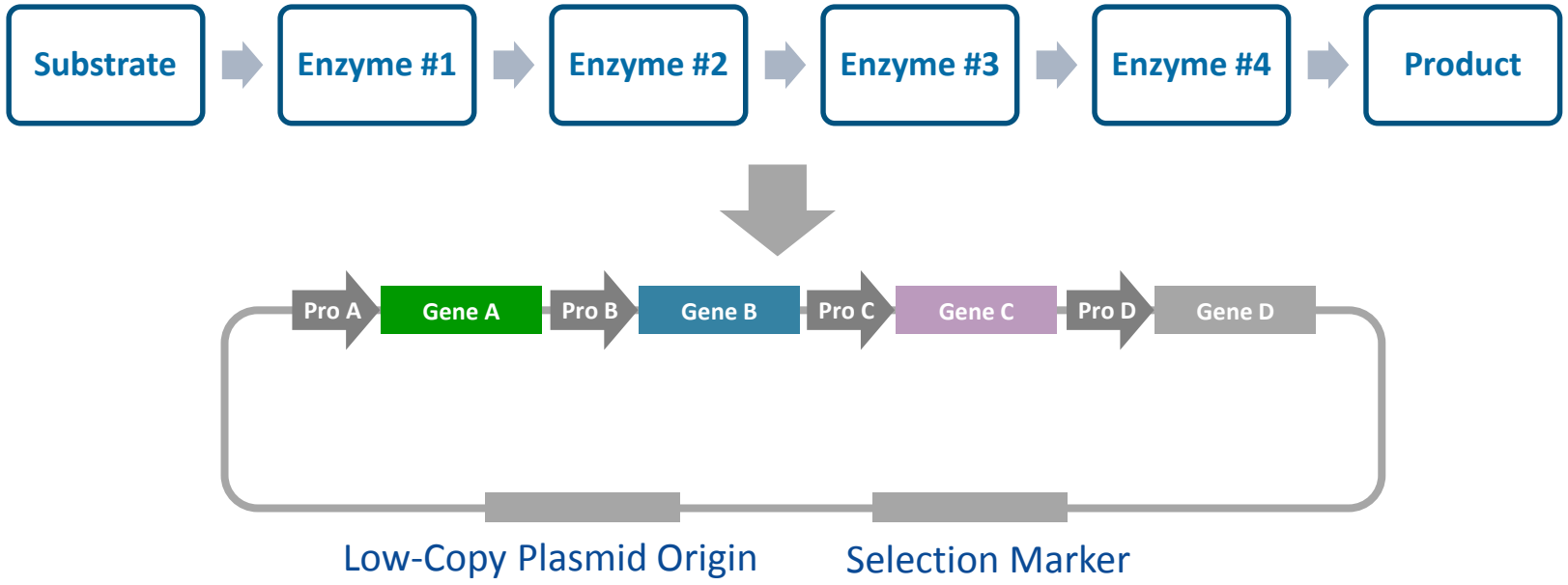


Case Studies for Combinatorial Optimization

Combinatorial DNA Library Services

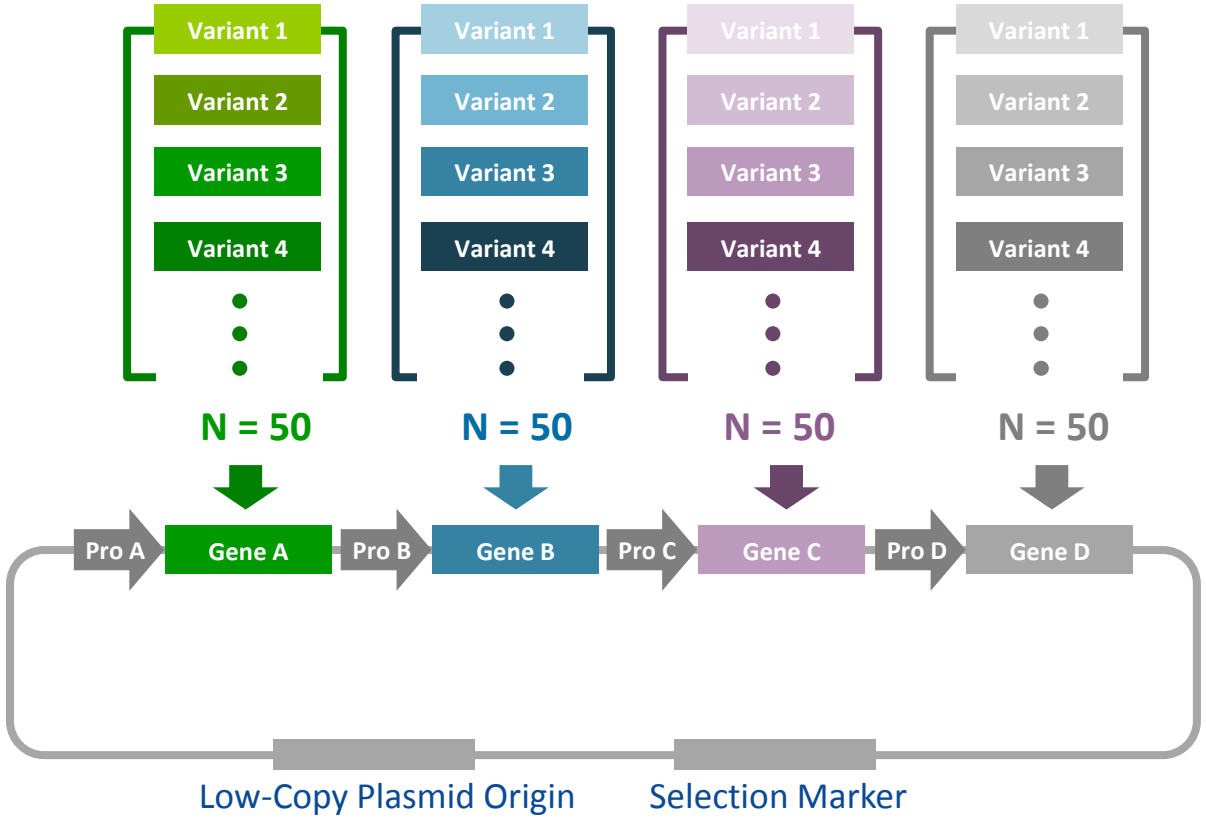
Case #1: Metabolic Pathway Design and Construction

Objective: Improving metabolite (*i.e.* product) yield



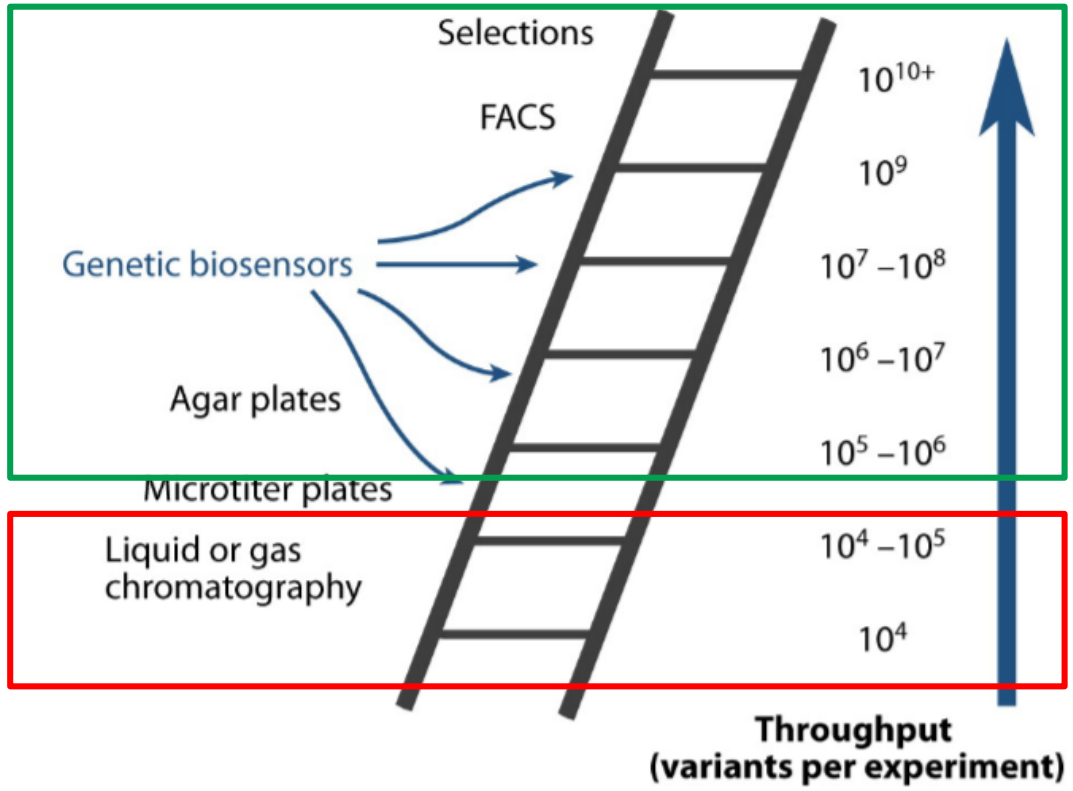
- Using yeast cells to produce the precursor for a small molecule drug
- Construct a four gene metabolic pathway in a low-copy number vector
- Using four constitutive promoters to efficiently express all four genes

Case #1: Pathway Optimization by Testing Different Enzyme Variants



- Identified 50 variants for each pathway gene through database blasting and literature search.
- Design: Random insertion of 50 gene variants in each CDS position in the pathway
- Theoretical library size: 6,250,000

Case #1: Throughput Screening for Different Testing Methods



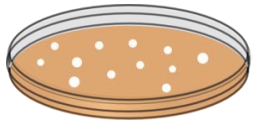
- Testing method: growth-based selection or visual selection of pigment
- Screening capacity: >10^6 library size
- Explore a large library size with pooled constructs.

- Testing method: chromatography
- Screening capacity: <10^3 library size
- Carefully design parameters in the project or use "Design of Experiment" to reduce library size.

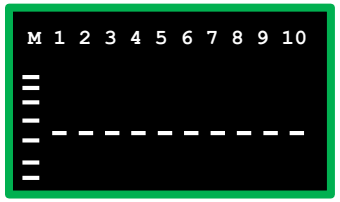
Annu Rev Biochem. 2010;79:563-90.

Case #1: Exploring Combinatorial Library by Randomly Picking Clones

Assembling combinatorial plasmid library as a pool

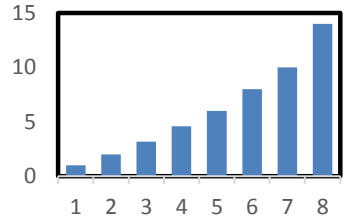


Transformation & plating in *E. coli*



Random colony picking & PCR verification

Plasmid mini-prep



Testing performance

Workflow

- Library construction as a pool – putting all fragments into the reaction
- Randomly tested 2,000 colonies; ~1600 of them have inserts, indicating an 80% positive rate.
- Mini-prepped all plasmids with HT platform, followed by transformation of plasmids into appropriate expression host to test performance.

Key Points:

- By not assembling and sequencing all combinations, lots of money and effort was saved.
- By testing a sub-library, enough time was saved for HPLC testing.
- Colony PCR verification ensures that every tested clone was positive (*i.e.* contained the designed components).

Case #1: Testing Library Diversity to Ensure Maximum Coverage

Combination #	Enzyme A#	Enzyme B#	Enzyme C#	Enzyme D#
1	45	25	40	19
2	8	24	16	30
3	49	37	32	6
4	9	41	31	30
5	36	10	22	8
6	44	13	37	4
7	15	44	14	2
8	23	28	4	13
9	15	44	12	20
10	13	12	29	47
11	32	37	3	40
12	36	18	31	5
13	29	1	3	17
14	21	23	27	22
15	2	28	36	25
16	38	27	35	45
17	4	28	50	35
18	40	32	35	35
19	4	16	18	9
20	18	8	39	4
21	33	10	28	7
22	48	40	40	44
23	31	34	16	37
24	21	6	22	9

Combination #	EnzymeA #	EnzymeB #	EnzymeC #	Enzyme D#
25	43	1	6	26
26	14	36	48	9
27	10	25	26	15
28	34	24	10	4
29	32	1	32	50
30	11	30	21	7
31	2	43	46	28
32	38	46	1	49
33	13	18	47	3
34	37	50	40	5
35	8	17	42	41
36	5	22	49	38
37	5	19	1	10
38	20	4	48	38
39	47	27	31	49
40	14	46	23	2
41	16	47	24	49
42	13	29	14	11
43	36	37	19	23
44	3	32	2	36
45	45	34	2	37
46	35	5	3	14
47	35	22	15	19
48	15	36	39	49

Analyzing the sequence of 48 randomly-picked clones demonstrated that 100% diversity was achieved.

Case #1: Screening for the Best Clones

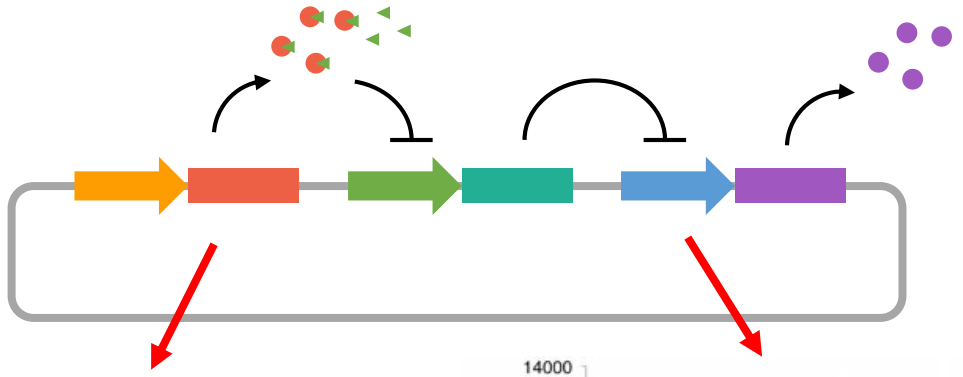
Top 24 Variants, Ranked by Production Level

Combination #	Enzyme A#	Enzyme B#	Enzyme C#	Enzyme D#	Production (Arbitrary unit)
1	13	49	11	23	
2	13	49	5	44	
3	10	46	11	17	
4	37	46	11	7	
5	26	42	11	44	
6	13	33	5	45	
7	44	44	27	40	
8	27	16	5	31	
9	14	46	38	9	
10	38	28	5	44	
11	49	42	30	46	
12	14	49	27	14	
13	37	16	5	6	
14	16	6	12	19	
15	13	49	11	45	
16	37	42	1	6	
17	32	36	39	23	
18	26	6	27	12	
19	37	42	4	20	
20	35	49	5	33	
21	42	17	22	31	
22	15	16	2	7	
23	3	34	3	8	
24	32	28	11	12	

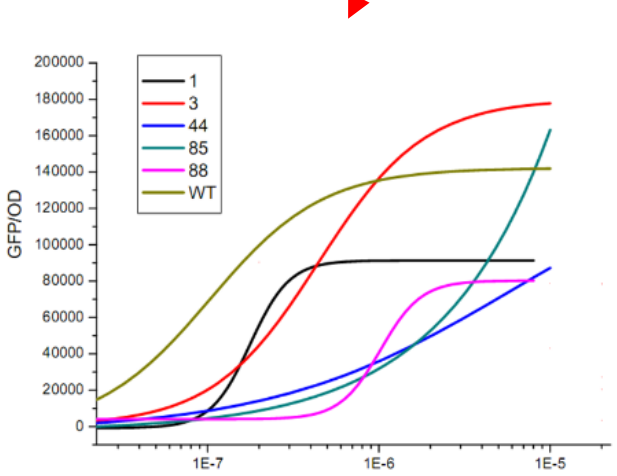
- Tested all 2000 randomly-picked clones with HPLC analysis.
- Sequence analyzed the top 96 variants. (Top 24 variants are listed here.)
- Some of the enzyme variants were highly enriched in the good-performing clones.
- These good-performing enzyme variants can be further combinatorically tested in the next round of optimization.

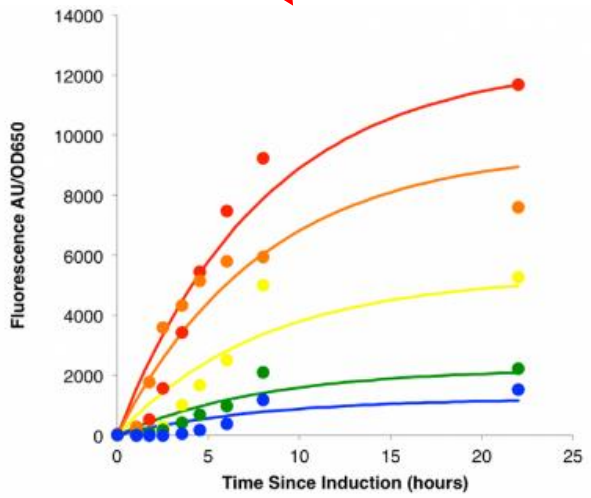
Case #2: Optimizing Gene Circuits for Therapeutic Applications

Objective: Production of a functional protein upon sensing specific environmental signals



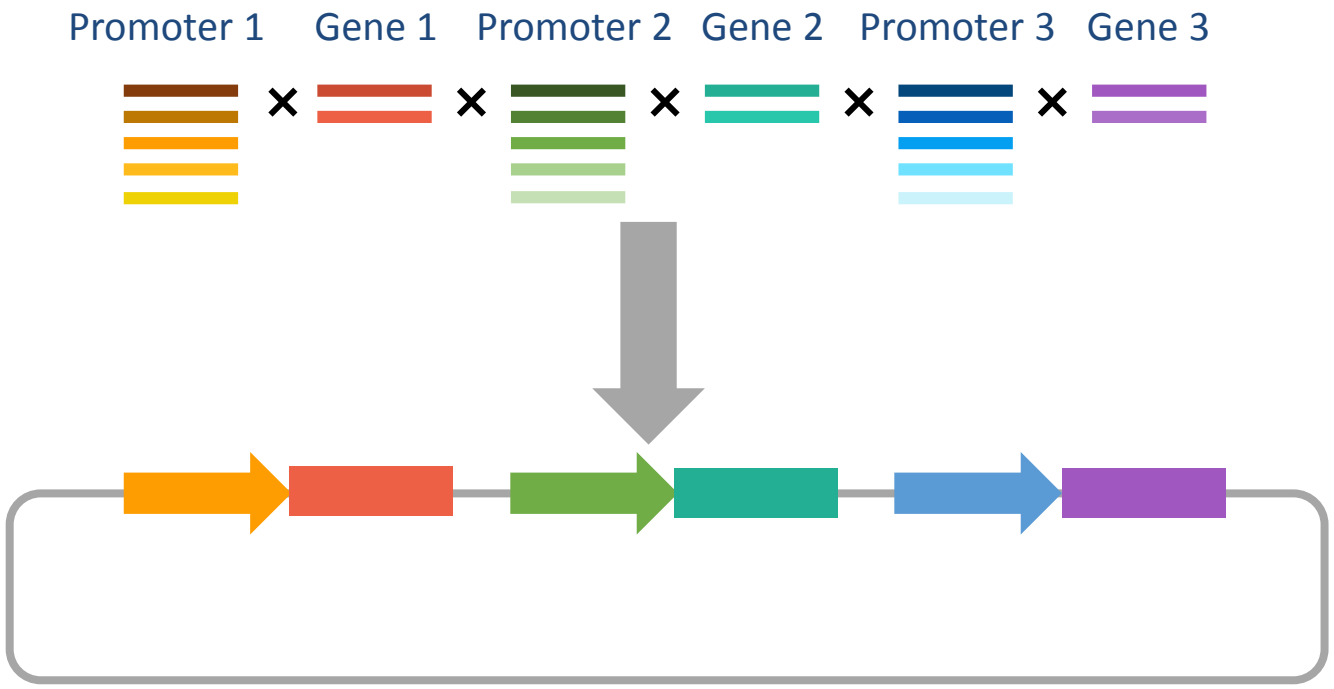
Different dose response curves for different regulators was needed.





Expression intensity could affect performance of the circuit.

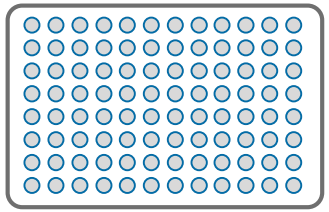
Case #2: Optimization of Gene Circuits



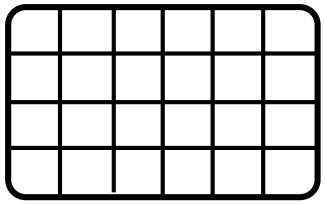
Project scope

- Testing five promoters with strength levels from very weak to very strong for gene expression
- Testing different versions of sensors and regulators
- Theoretical combination number: 5*2*5*2*5*2 = 1,000

Case #2: Construction & Testing of Each Combination Individually

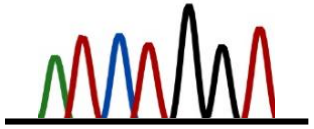


Building each construct individually in high-throughput format

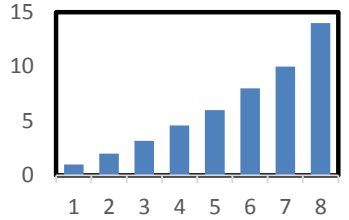


High-throughput PCR screening

Plasmid mini prep



High-throughput sequencing



Testing performance

Workflow:

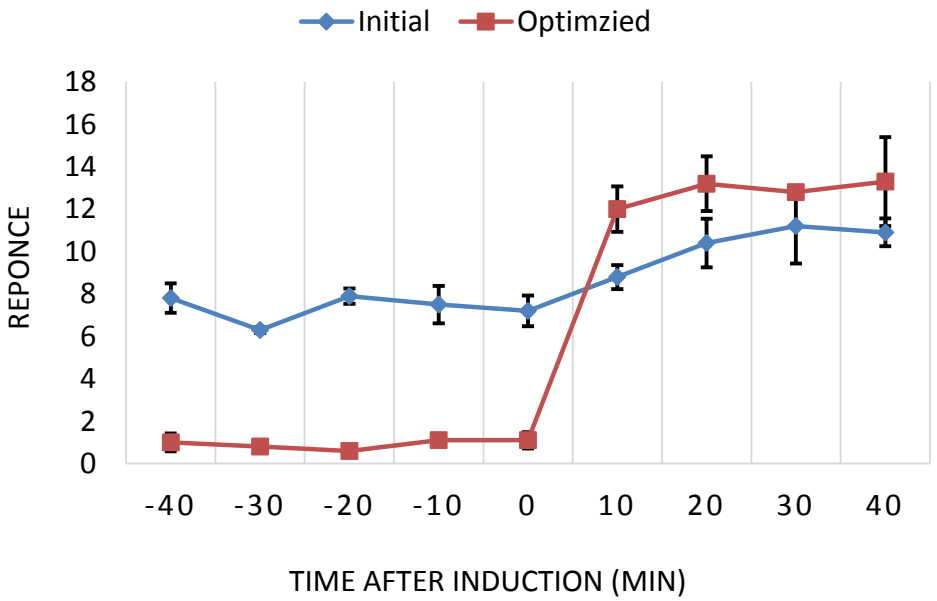
- Assembled 1,000 construct individually and acquired 943 perfect clone in the first run.
- Constructed the remaining 57 clones in the second run.

Key Points:

- Individual building and testing of every construct allowed for direct evaluation of each construct.
- Suitable for thoroughly explore the combinatorial design space
- Compatible with “Design of Experiment”

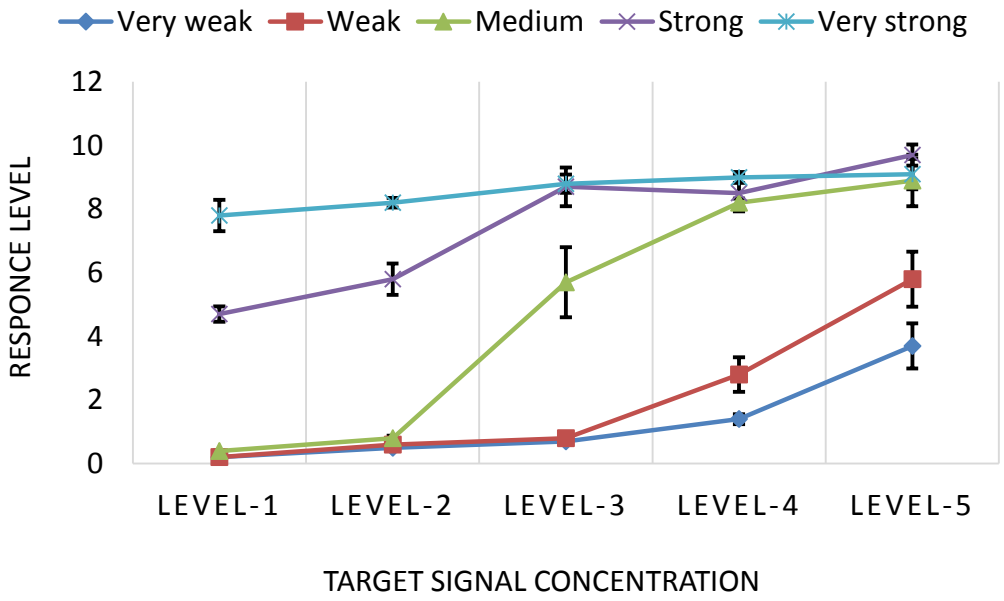
Case #2: Optimized Circuit exhibited Improved Performance

Response Curve after Induction



The optimized circuit exhibited stronger and more prompt response behavior than the initial.

Inducer Dose Response for Promoter-3 Strength



The medium-strength promoter-3 showed the best dose-response behavior.

Summary of Case Studies

Case #1

Representative Combinatorial DNA Library

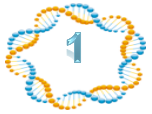
- Library construction as a pool
- Random colony-picking for downstream testing
- Saves a lot of money and effort
- Suited for the initial phase of biological optimization

Case #2

Arrayed Combinatorial DNA Library

- Building and testing of every construct individually
- Suitable for thoroughly exploring the combinatorial design space
- Specifically suitable for optimizing non-linear systems
- Compatible with “Design of Experiment”

Outline



Combinatorial Optimization for Metabolic Pathway Engineering

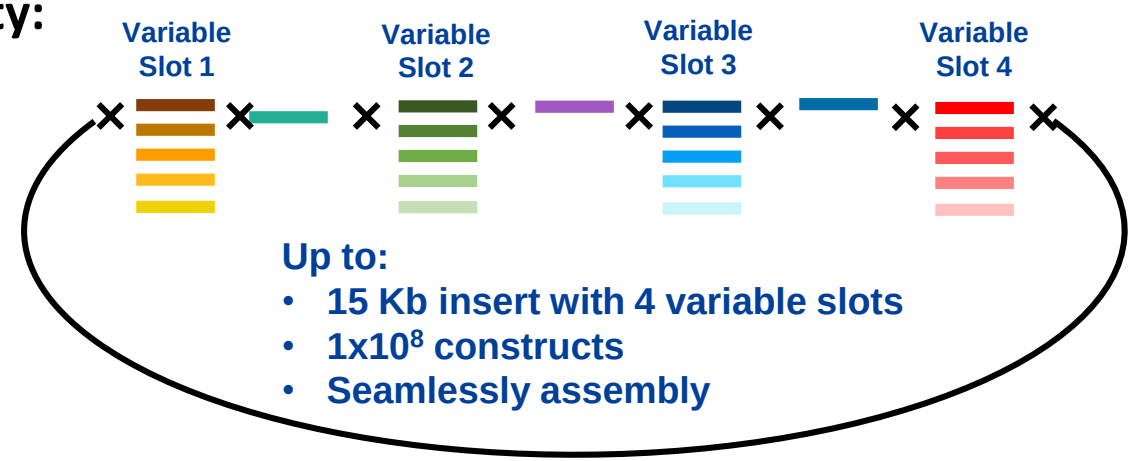
Case Studies for Combinatorial Optimization

Combinatorial DNA Library Services

Combinatorial DNA Libraries

Providing a **highly customizable** approach for **accelerating** the **build phase** of your **metabolic pathway** and **microbial strain engineering process!**

Our Capability:

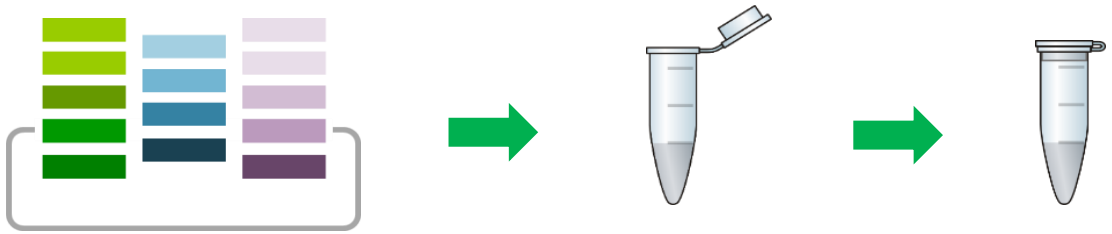


Applications:

- ***Metabolic Pathway & Microbial Strain Engineering***
- ***Gene Expression Regulation***
- ***Protein & Antibody Engineering***

Combinatorial DNA Library Services

Pooled Combinatorial DNA Library



Assembling combinatorial plasmid library

Constructing plasmid library as a pool

Delivering as pooled plasmids

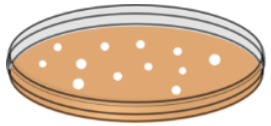
Ideal for	• > 10^4 throughput screening • For screening platforms that are not sensitive to the presence of negative clones
Service Features	• Cloned into pUC57 or custom vector with 4 μg in quantity • Delivering pooled a plasmid library with up to 1×10^8 library size
QC Standards	• PCR verification of more than 48 clones to determine positive rate • Sequence verification of 24 positive clones with a guarantee on more than 85% diversity

Combinatorial DNA Libraries

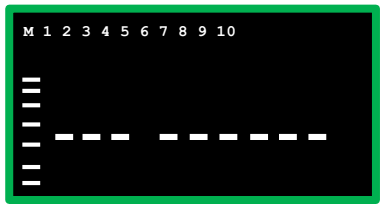
Representative Combinatorial DNA Library

Assembling combinatorial plasmid library

Constructing Plasmid library as a pool



Transformation



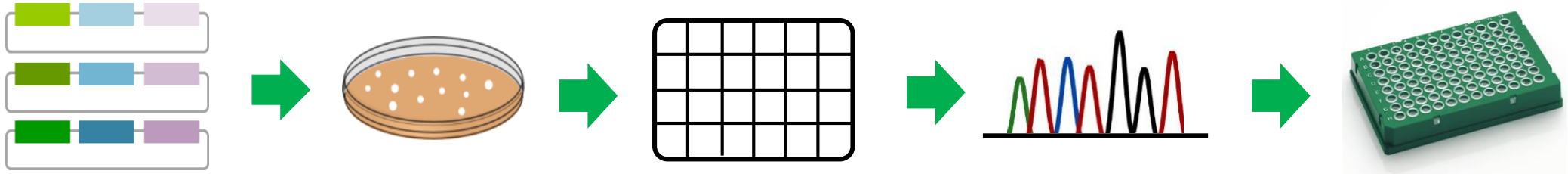
Random colony picking and PCR verification

Delivering randomly-picked & PCR-verified individual plasmids

Ideal for	• 10^2 - 10^4 throughput screening • Screening a pool with no concern for the presence of exact sequence in each clone
Service Features	• Guaranteeing that every delivered construct contains all designed parts or modules • Delivering up to 10,000 randomly-picked and PCR-verified individual constructs • Cloned into pUC57 or custom vector with 4 µg in quantity
QC Standards	• PCR verification of all delivered plasmids • Sequence verification of 24 positive clones with a guarantee on more than 85% diversity

Combinatorial DNA Libraries

Arrayed Combinatorial DNA Library

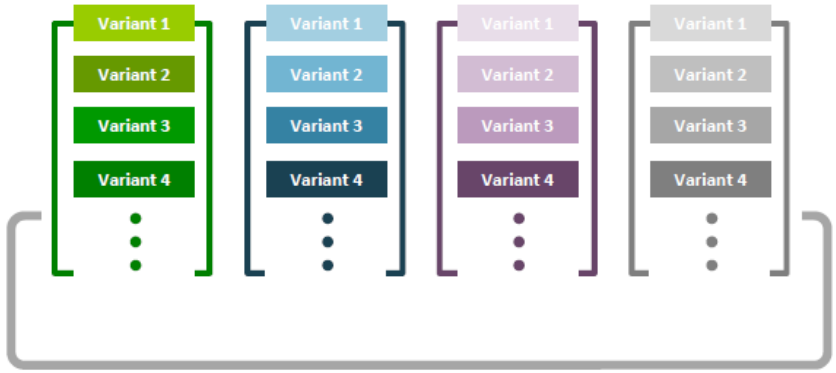


Each insert is individually constructed → Transformation → High-throughput PCR screening → High-throughput sequencing → Delivery as sequence-verified individual constructs

Ideal for	Testing every single design in your library
Service Features	• Enabling the design of every construct in the library in any combination • Delivering up to 10,000 sequence-verified individual constructs ready for transformation • Cloned into pUC57 or custom vector with 4 μg in quantity
QC Standards	Each delivered plasmid is sequence-verified.

Combinatorial DNA Libraries: Advantages

Let DNA Building Experts Speed Up Your Metabolic Pathway and Microbial Strain Engineering Process!



- ✓ **One-stop, high-throughput** solution
- ✓ **Highly-customizable**
  - In pool or individual formats
  - Up to $1x10^8$ constructs (pooled library)
  - Up to 4 variable slots for 15 kb inserts
- ✓ **Faster** and **more economical** compared to your in-house operations
- ✓ **Seamlessly assembled** with advanced methods
- ✓ **Expert advising** on all project plans by our Ph.D.-level scientists

Thank you!

For any questions,

please visit:
www.genscript.com/combinatorial-DNA-library.html

or email:
clairez@genscript.com
gene@genscript.com