Combinatorial DNA part assembly for large-scale pathway library construction

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ABSTRACT

In multi-gene metabolic pathways, the expression levels of each gene can substantially influence the overall performance of the synthetic system. It is well known that optimal performance is achieved by the appropriate expression ratio of the genes. Several techniques have been established to identify the optimal ratio of gene expressions by investigating a broad spectrum of their expressions. However, due to the exponential increment of combinations as the number of genes increases in a pathway, traditional assembly techniques are difficult to cover all the possible combinations. This study presents a combinatorial DNA part assembly approach by modifying GoldenBraid assembly and mutagenesis PCR. While GoldenBraid uses two enzymes and two vectors, our approach enables to continuously assemble DNA modules from scratch DNA part to long pathway using one enzyme and vector. We confirmed the DNA part combinatorial ratios are constantly remained in each assembly step using the long-read sequencing technique. We anticipated that this approach is helpful to optimize multi-gene pathway systems by constructing a large-scale pathway library with a high-throughput screening system.

Combinatorial DNA part assembly

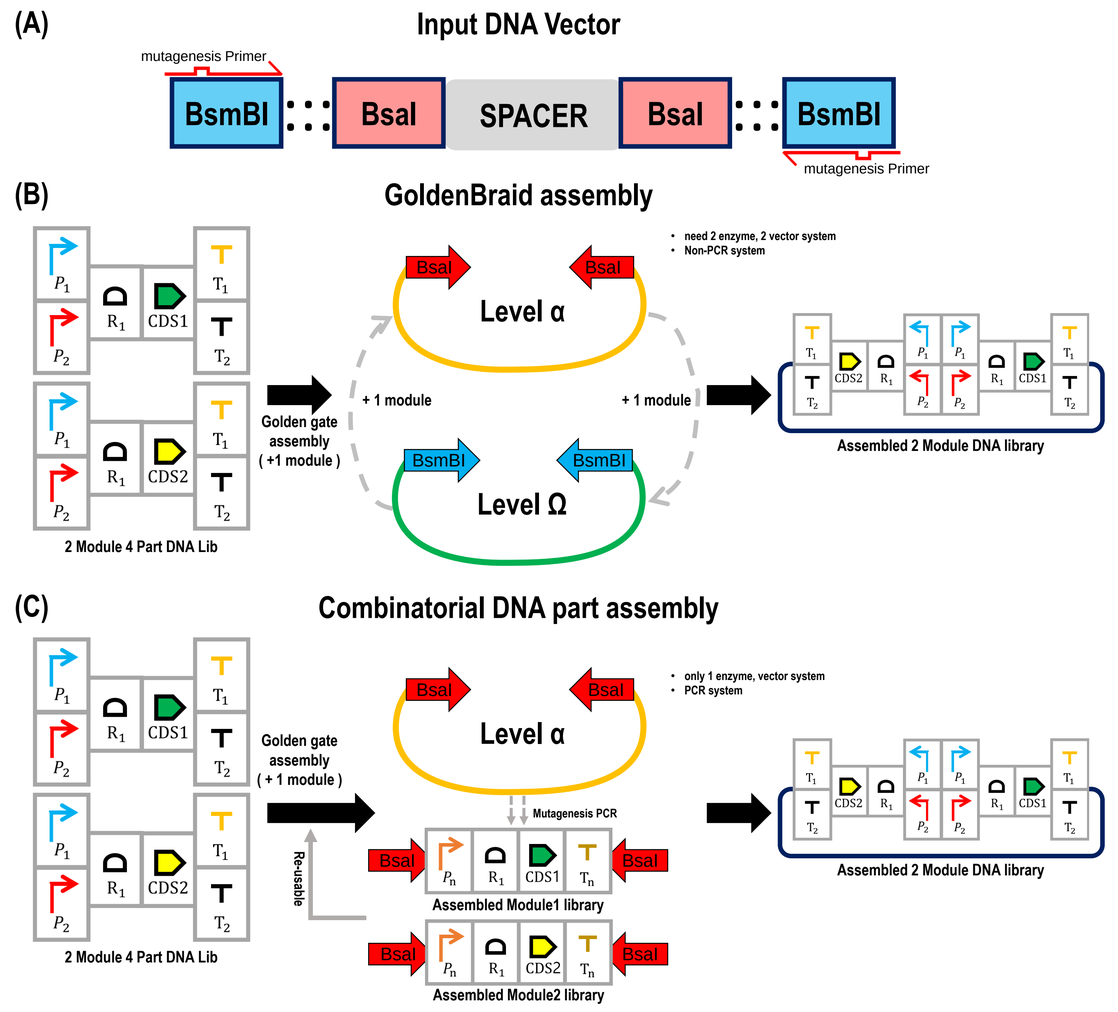


Fig 1. Combinatorial DNA part assembly
(A) Description of Level α vector used in this method
(B) GoldenBraid assembly, An assembly method that assembles DNA part using two types of enzyme and vector system
(C) Combinatorial DNA part assembly : Continuously assembly method, using a Level α vector(A) from GoldenBraid and one enzyme. first, assemble the scratch DNA part library with golden gate assembly. second, regenerate BsaI enzyme site with mutagenesis PCR. third re-use the PCR product as Input of next golden gate assembly to construct large-scale DNA library
* P= promoter, R= RBS, T= terminator

DNA Part combinatorial ratio confirmation

Using, long-read sequencing technique
confirmed that only very little bias occurred
on the DNA part combinatorial ratio
during the whole combinatorial DNA part assembly process.

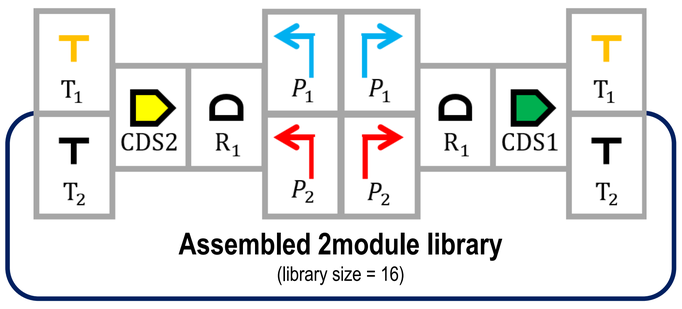
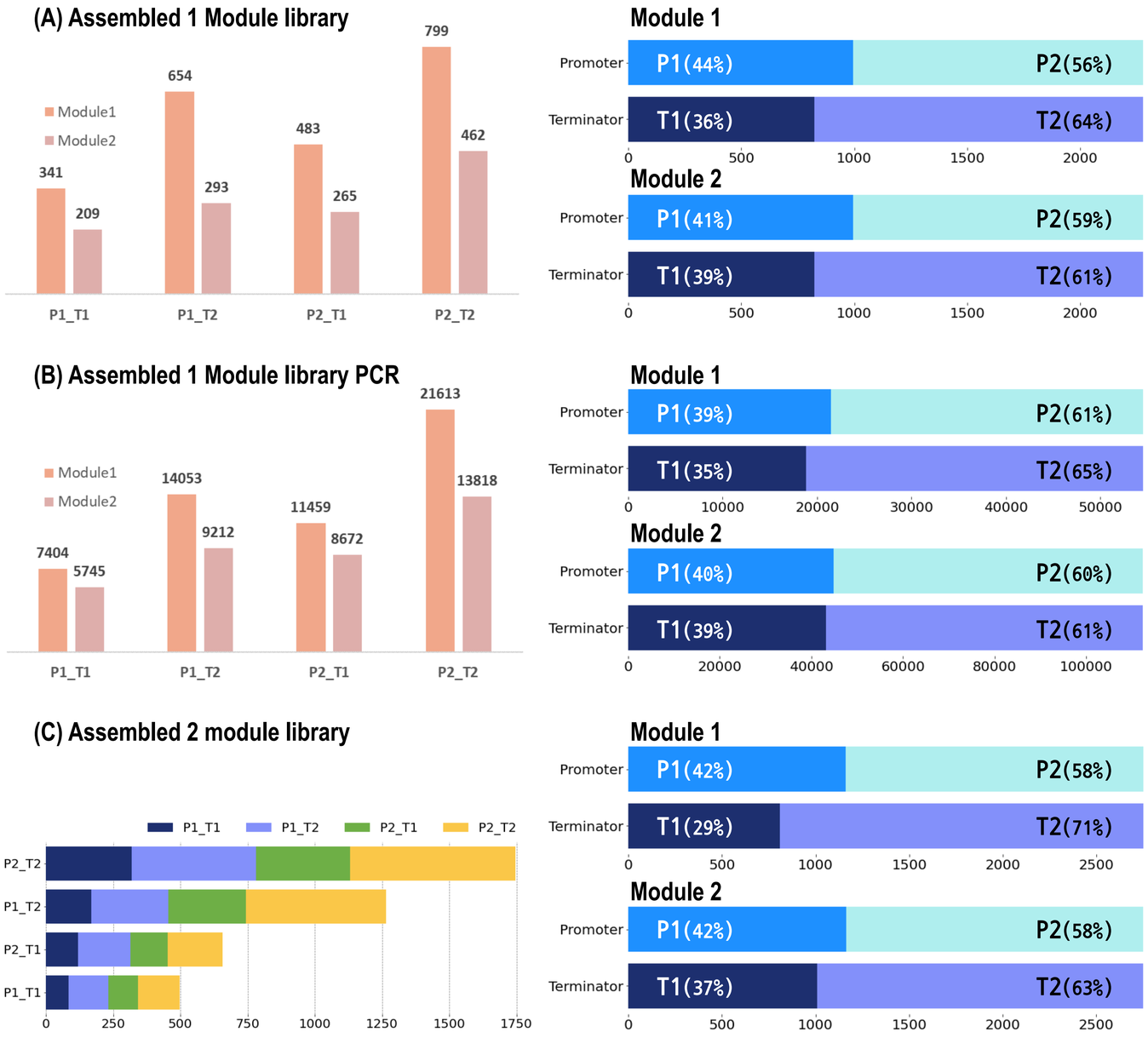


Fig 2. Combinatorial DNA part ratio confirmation
In each reaction of combinatorial part assembly, confirm every combinatorial DNA library ratio using Oxpord Nanopore long-read sequencing
(A) Assembled 1 module library, product of first golden gate assembly with scratch DNA part
(B) Assembled 1 module PCR library, product of mutagenesis PCR to regenerate enzyme site with (A) sample
(C) Assembled 2 module library, product of second golden gate assembly with (B) sample
* Among the sequencing reads, only 90% or more querycover reads were selected and calculated.



Conclusion & Discussion

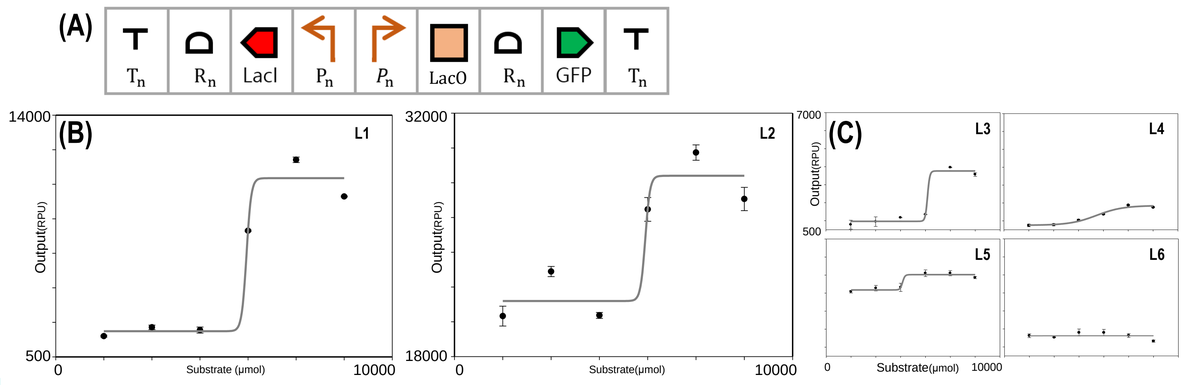


Fig 3. Combinatorial genetic circuit characterization
genetic circuit library was constructed and characterized by the method of fig 1. it was confirmed that circuits with different features has been completed, and it had a large correlation with the inserted DNA part
(A) simple diagram of LacI-GFP genetic circuit
(B) Among the discovered circuit, a circuit with a particularly high dynamic range and low LOD was found.
(C) circuits with different characteristics separated from one assembly

- Unlike GoldenBraid assembly, it is possible to efficiently construct large-scale DNA library by using 1 enzyme and vector system.
- Even while using PCR, it has been confirmed that bias does not occur severely, and there is a certain advantage when making large-scale combinatorial DNA library.
- Using the above method, numerous circuits that exhibit different characteristics due to different DNA part were found. This suggests it is helpful enough to construct the optimal large-scale pathway.
- In this study, we assembled a relatively large number of parts at once, however we couldn't confirm the efficiency even after it was repeated several times

References

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Acknowledgments

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