# High-throughput bio-part characterization using combinatorial library assembly approach

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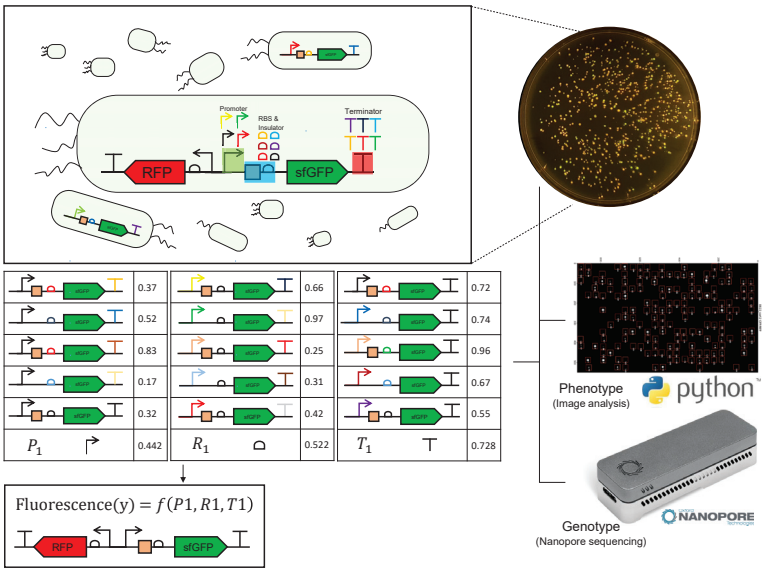
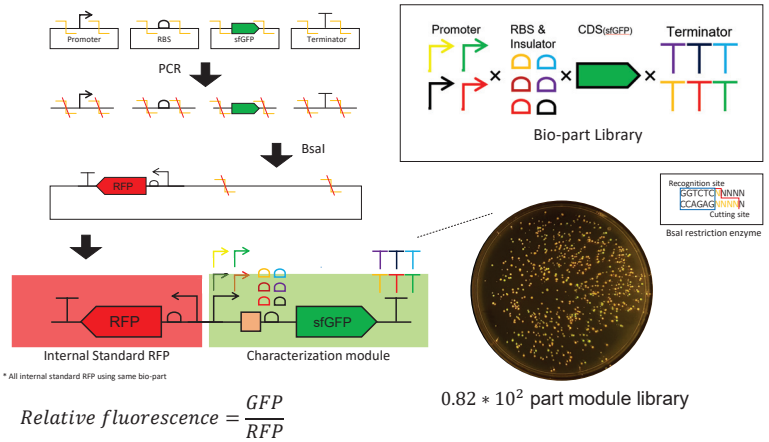
## ABSTRACT

For the successful design of a genetic circuit, simulation techniques are required for selecting an appropriate circuit that shows the desired output among various combinations of parts. The prediction technique reduces the time and effort required for design, build, and test unnecessary part combinations. To do this, quantitatively characterized bio-parts are essential. In conventional methods to quantify the parts, the strength of every part is measured by the expression ratio of green fluorescence protein involving the target part, and the reference red fluorescent protein. However, this approach has a problem in that the measured quantity can be varied depending on the combination of other parts in the same circuit. Also, the quantification process takes time as the number of parts increases. In this study, we built the combinatorial library of parts using the Golden gate assembly technique and quantified the parts in the combinatorial library by measuring their fluorescence in the colony state. The genotype of the colonies was obtained by a long-read sequencing technique using tagging primer. The proposed technique can calculate quantitative values of the DNA parts considering the interaction among the parts, and quantify multiple parts in a high-throughput manner with a single experiment with combinatorial libraries.

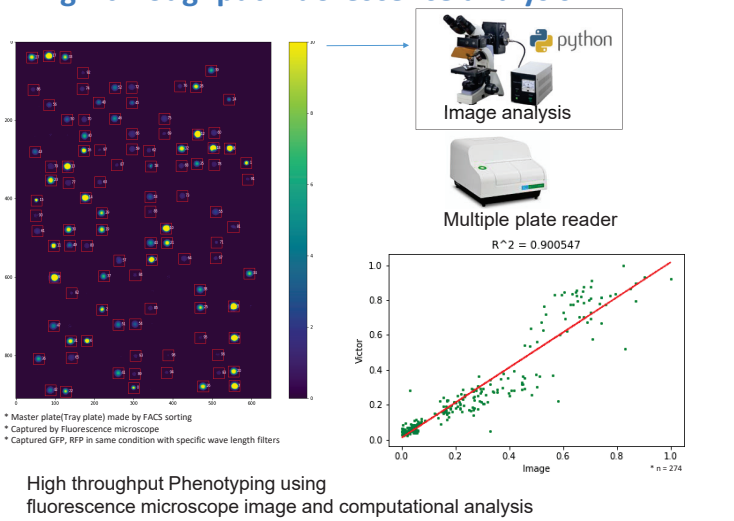
## Introduction




## Construction of Bio-part Library

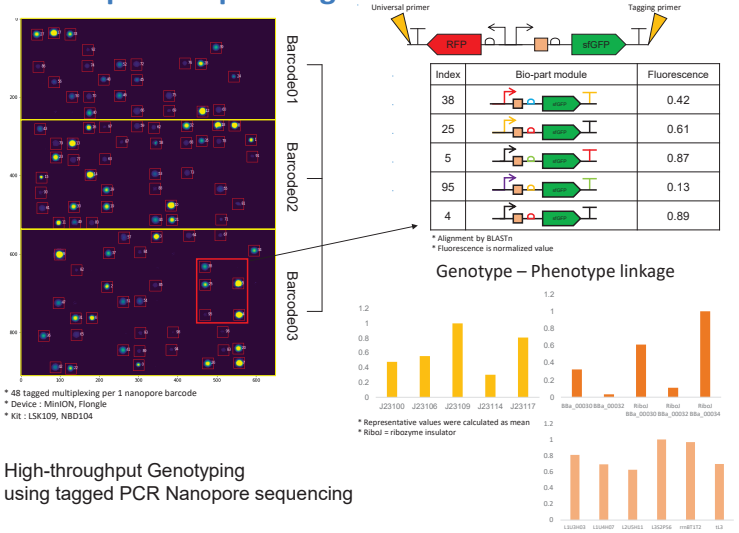



## High-throughput Fluorescence analysis



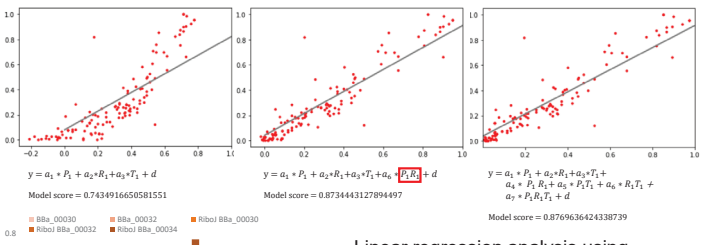

High throughput Phenotyping using fluorescence microscope image and computational analysis

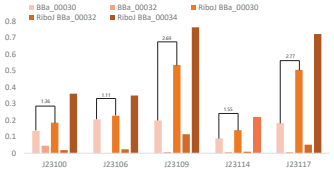
## Nanopore sequencing




High-throughput Genotyping using tagged PCR Nanopore sequencing

## Conclusions & Discussion




Linear regression analysis using representative values confirmed the Interaction between specific Promoter and RiboJ

* y = relative fluorescence
* The additional line is Fold-change

- $0.82*10^2$part module library constructed by golden gate assembly.
- We confirmed the correlation between computational Image analysis and multiple plate reader.
- Representative bio-part value is measured as mean of variable library.
- Using linear regression analysis confirmed the interaction between specific Promoter and RBS with insulator RiboJ
- For extended characterization, Two or three module circuit should be considered

## References

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2. Nuñez I, Matute T, Herrera R, et al. Low cost and open source multi-fluorescence imaging system for teaching and research in biology and bioengineering. PLoS One. 2017;12(11):e0187163. Published 2017 Nov 15. doi:10.1371/journal.pone.0187163
3. Registry of Standard Biological Parts[http://parts.igem.org]

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