Flexible Automated Platform for the Assembly & Test of Recombinase State Machine-Based Genetic Circuits

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Introduction

Unique DNA recombinase recognition site configurations control gene expression through logical excision and inversion of DNA. Based on a set of chemical inputs, recombinase state machine, or RSM, based genetic circuits predictably transition between states. When designed to form a memory circuit, RSMs can track a cell's temporal changes during mutation, differentiation, and gene expression. The typical design and assembly of RSMs is time consuming and prone to low success rates. Through the design of standardized and automated register assembly, RSM-based genetic circuits increase in scalability and ease-of-use.

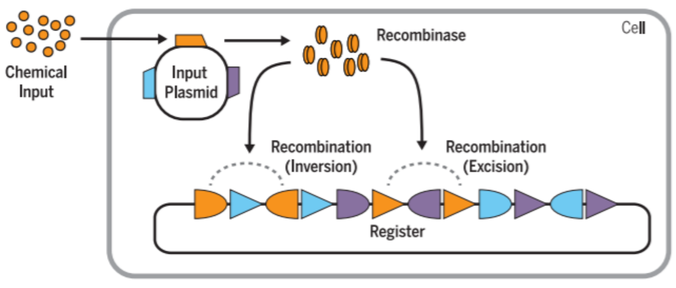

Figure 1: Example configuration of an RSM register supplied by Roquet *et al.* displaying excision and inversion.

Summary

Our standardized RSM assembly pipeline explores both Gibson (Synthetic Genomics) and BASIC (Imperial College-REF) methodologies utilizing the OT-2 (Opentrons) low-cost liquid handling robot. Both Gibson and BASIC assembly methods involve two-tier, double antibiotic selection with tier-one optimized, reusable assemblies. Six two input, five-state machines will be constructed using both methodologies, each with three unique registers of varying sizes containing green and blue fluorescent protein (G/BFP) coding sequences. The first state machine assembly, accomplished via unique nucleotide sequence (UNS)-guided Gibson, returned successful flow cytometry results with expected GFP expression in state four and BFP expression in state five. Leaky GFP expression in states three and five was present and expected. Further assembly standardization requires (i) BsaI-site point mutations and characterization to allow for BASIC constructs, (ii) part library construction, and (iii) accompanying software tool development for automated assembly. With this modular and automated pipeline established, we will provide tools to facilitate RSM use in exploring order and timing in cellular processes.

Methods

Experimental Design

For full platform applicability, 18 RSM input plasmids will be constructed comprised of six state machines, each with three unique register configurations of varying length (short, medium, long).

Methodologies:

- Gibson and BASIC
- Manual and Automated (OT-2)

Considerations:

- Mutation of existing BsaI sites
- Efficiency with bacterial artificial chromosome

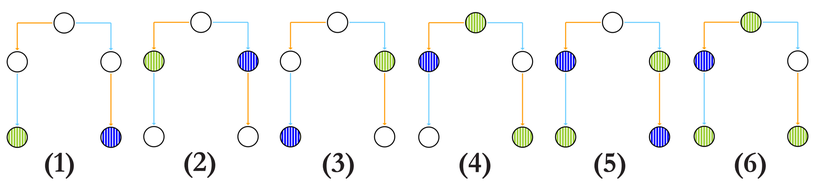

Figure 2: Six chosen RSMs. Each RSM was selected to maximize differentiability during flow cytometry without necessitating sequencing. RSM1 has been constructed and is being utilized to test BsaI mutation variants.

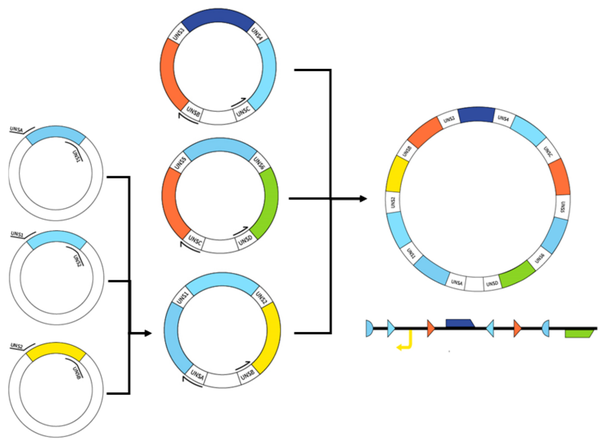

Figure 3: UNS-guided 2-tier Gibson assembly.

Software Design

Currently available at http://34.212.236.86:5000/.

Features:

- Selection of genes from SynBioHub
- Output of GenBank file based on RSM input

Future Direction:

- Selection of backbone from SynBioHub
- Integration with automated instructions

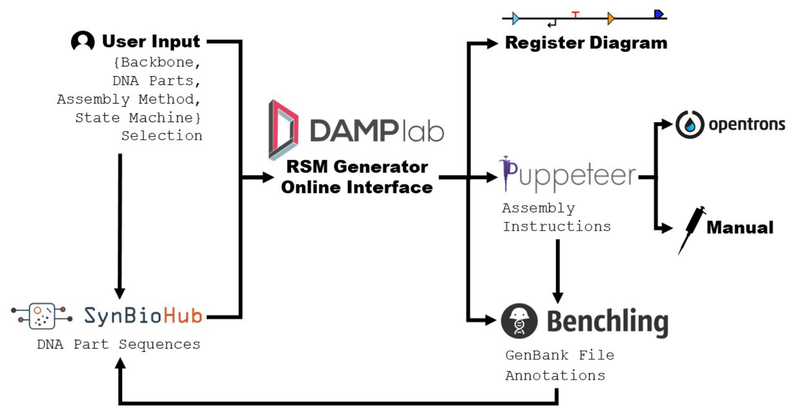

Figure 4: Software diagram detailing intended inputs and outputs of the RSM generator.

Results

State machine #1 (shown in *Figure 2)*, register #1 (short, 10 parts) was constructed via Gibson and verified. The flow cytometry results were consistent with the expected expression, including leaky GFP expression in states three and five found in Roquet *et al.*

State	Expected DNA Register	Output (Expected)	Output (Measured)	GFP %	BFP %
S1		Null	Null	0.17	0.53
S2		Null	Null	1.07	0.10
S3		Null	Leaky GFP	2.89	0.42
S4		GFP	GFP	82.70	0.26
S5		BFP	BFP	19.53	73.87

Table 1: Expected and measured flow cytometry results after chemical induction for RSM1 states 1-5.

Conclusions & Future Work

Increasing feasibility of RSM-based genetic circuit construction will facilitate increased use. Based on the primary results of the first RSM assembly, the integration of UNS spacers does not impact the expected functionality of the genetic circuit, allowing for UNS-guided Gibson assembly. Anticipated completion of this project is the summer of 2019.

Next Steps:

- Select part variants based on BsaI-site point mutation results
- Construct library (10 unique parts)
- Add backbone selection feature
- Construct RSM1 register 1 using two-tiered Gibson and BASIC assemblies
- Quantify cost and time differences between automated and manual assembly

Acknowledgments

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References

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