Compositional Genetic Inverter Circuits for Complex Logic Functions

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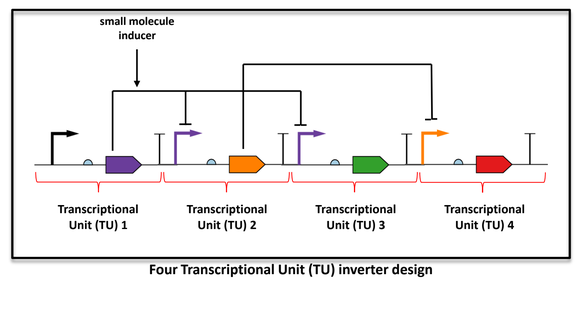
INTRODUCTION

Minimally, a transcriptional inverter (NOT-gate) consists of an inducibly-expressed repressor and its cognate repressible promoter driving the expression of some measurable output. We wish to expand our designs to conduct a more thorough investigation of the design space and for improved inverter behaviour.

1) We wish to build **the best possible inverter from our available regulatory part design space** of 31 constitutive promoters, 5 RBSes, 4 repressor-repressible promoter pairs and 2 inducer-inducible promoter pairs. However, the number of possible inverters is very large to build and test each one. **We define a good inverter as having high RFP and no GFP before induction, GFP expression directly proportional to induction level, no RFP and highest GFP at maximum induction and maximum separation in GFP and RFP expression levels at no induction and maximal induction.**

2) We **propose a streamlined workflow** for systematically building genetic circuits using a combination of software tools to refine our design space, automate assembly plan and multiplex circuit synthesis.



WORKFLOW AND METHODS

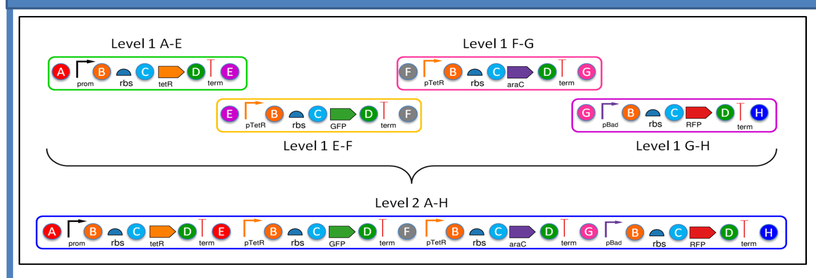


Figure 1 (above): New inverter design using the standardized Modular Cloning (MoClo) library developed by the Densmore lab and the 2012 BU iGEM team.
A full inverter is a 16-part construct consisting of four 4-part transcriptional units (TUs). Each transcriptional unit is assembled in a one-pot Level 1 reaction. Type IIs restriction enzyme-ligase reaction through the fusion of complimentary overhangs left by Type IIs restriction enzyme cleavage. Four Level 1 TUs are subsequently assembled by a different one-pot Level 2 reaction to assemble a complete inverter.

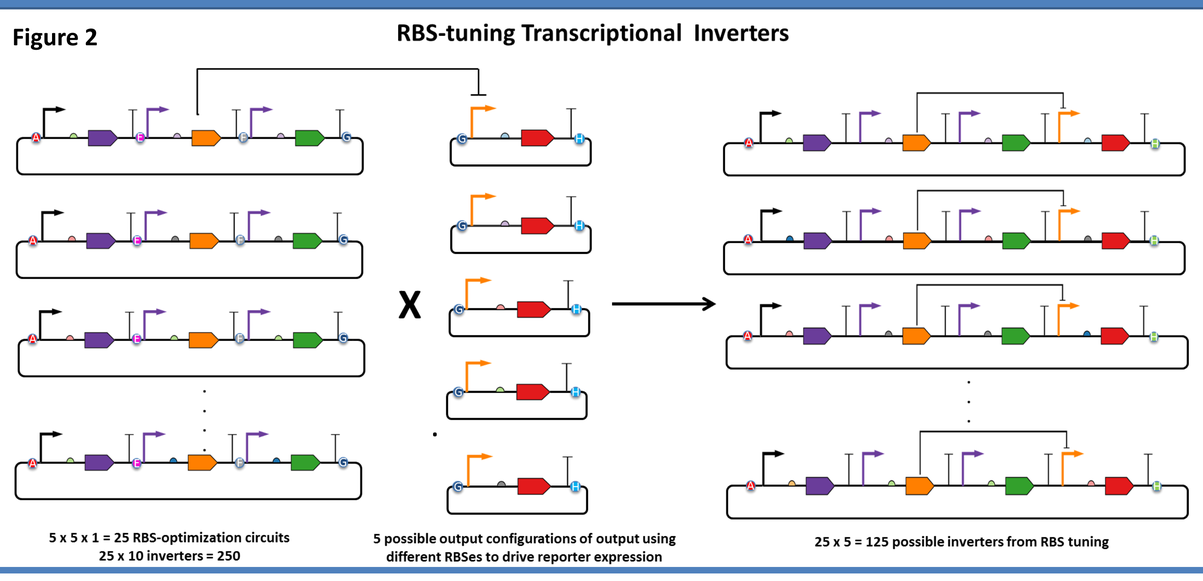


Figure 2: RBS tuning of Transcriptional Inverters.

To tune the RBSes for optimal function, we build each of the four intermediate transcription units (TUs) with each of 5 RBSes of varying strength. TU-3 expressing the GFP reporter is constrained to always contain the same RBS as TU-2 expressing the repressor protein. This allows for 5 x 5 x 1 = 25 possibilities. TUs 1-3 are assembled into an intermediate construct Repressor Characterization Device (RCD) to optimize the relationship between induction level and repressor-reporter expression. Any RCD and output TU can be optimized by co-transformation and testing for reporter expression.

RESULTS

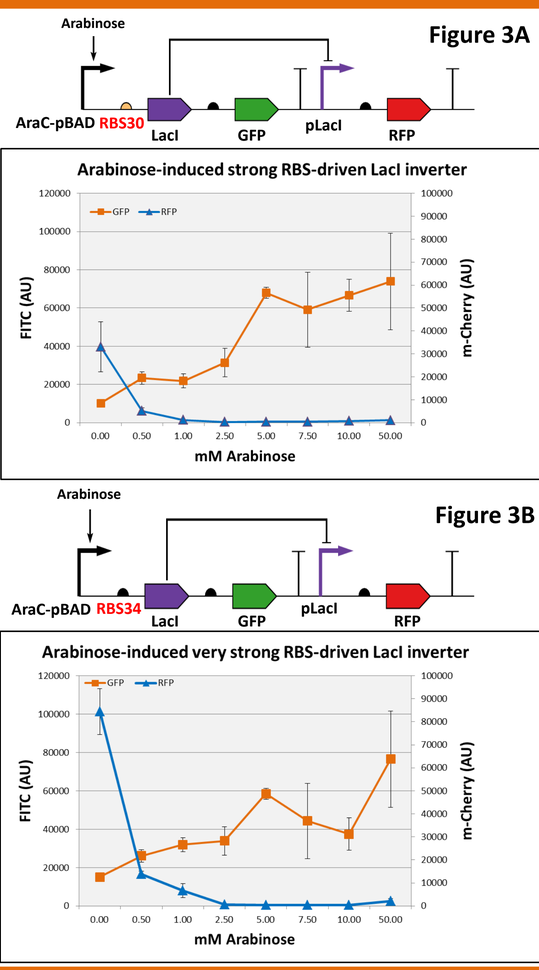


Figure 3A

Figure 3B

Figure 3: Arabinose induced-LacI inverters where the LacI repressor is being driven by BioBricks RBSes of different strengths: strong RBS B0030 and strongest RBS B0034 respectively. All other RBSes are the strongest RBS B0034. Each inverter was tested in triplicate. In the absence of arabinose, GFP expression should be at its lowest level and RFP expression at its highest. As the inverter is induced, repressor LacI and GFP are expressed. LacI represses LacI which inhibits RFP expression.

Figure 3A: Although inversion of expression is clear, the difference in RFP and GFP levels prior to induction is much smaller than the difference in their levels at full induction.

Figure 3B: Changing the RBS driving the expression of repressor LacI leads to better inverter behaviour. The difference in RFP and GFP levels before induction are greater that that in the above inverter in figure 3A.

New Design

```
Transcriptional Inverter

//Properties
Property Name(txt);
Property Represses (txt);
⋮
Property Repressedby (txt)

//Properties
PartType InduciblePromoter (Name, Sequence, Inducedby);
PartType Inducer (Name, Sequence, Induces);
⋮
PartType Terminator (Name, Sequence);

// Inverter Description
Device Inverter =
   ConstPr + RBS + Repressor + Terminator +
   RepPr + RBS + Repressor + Terminator +
   RepPr + RBS + Reporter + Terminator +
   RepPr + RBS + Reporter + Terminator;

// Represses relationship
Inverter[2].Represses EQUALS Inverter[4].Name
Inverter[2].Represses EQUALS Inverter[8].Name
Inverter[6].Represses EQUALS Inverter[12].Name
// RBSs
Inverter[5] EQUALS Inverter[9]
// Reporters
E0040 WITH E1010m AND E1010m AFTER E0040
// Repressors
Inverter[2] NOTEQUALS Inverter[6]

Size                16
Possible Designs    10,368
Rules               7
Valid Designs       216
Run Time            0.45sec
```

Build

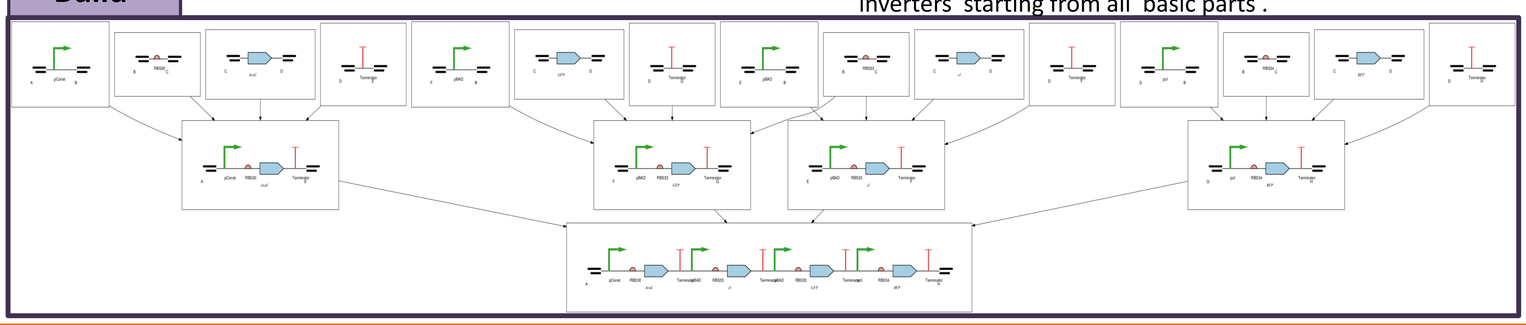


Figure 4 (left, top): Automated exploration of the design space of transcriptional inverters by the design specification language Eugene.
Eugene is a human and machine-readable language that provides users with a list of possible designs based on a given specification derived from a collection of, parts, part- types that capture part-relationships and behaviours as well as constraints in the form of rules provided to it.

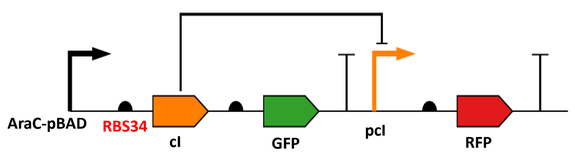
Figure 5 (left, bottom): Automated assembly plan generated by Raven.

Raven is a tool that intuitively designs an assembly plan using any of the most popular cloning strategies currently in use. Raven can take Eugene designs as its inputs and provide a detailed assembly plan optimized for either cost, speed or efficient use of existing intermediates.

Here we show a Raven design for our new transcriptional that we want to make using our standardized MoClo assembly inverters starting from all basic parts .

RESULTS

Figure 6



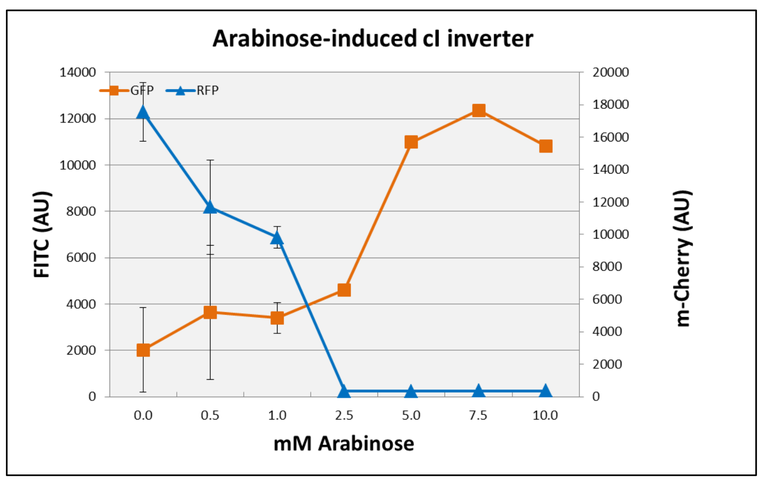


Figure 6: Arabinose-induced cI inverter.

All RBSes are the strongest BioBricks RBS B0034.

Prior to induction with arabinose GFP levels should be zero and RFP expression should be at maximum. As inducer arabinose is added, repressor cI and GFP are produced leading to an increase in GFP and a decrease in RFP levels.

Although we see a large separation between GFP and RFP levels at zero and maximum arabinose levels, GFP appears to be present prior to induction so further modulation of the component parts is necessary.

Furthermore, at the concentration of arabinose at which RFP expression is already zero, GFP is only moderately expressed, which suggests further modulation of design is necessary.

FUTURE DIRECTIONS

Figure 7

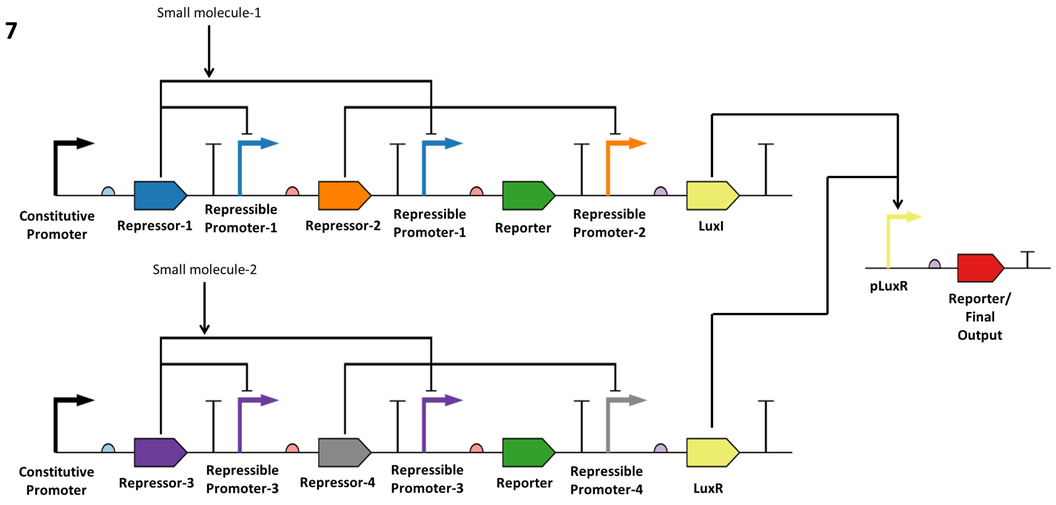


Figure 7: NAND-gate constructed from genetic inverters

Once we have built and optimized our library of inverters we wish to use them to build larger devices such as NOR and NAND gates. A NOR gate can be constructed by adding a second inducible promoter or an input module upstream of an inverter circuit.
Here we show two distinct inverters with separate inputs and repressor molecules. One inverter produces LuxI, the precursor to the intercellular signalling molecule, AHL and the other produces LuxR, the allosteric regulator for AHL-base signalling. The final output is the reporter RFP driven by the AHL-LuxR responsive promoter pLuxR. Our goal is to use this NAND circuit for optimizing signal routing and cell-cell communication inside a microfluidic device.

ACKNOWLEDGEMENTS

Genetic Parts and Devices images made with Pigeon (Bhatia and Densmore, 2013; http://pigeoncad.org/). Eugene design made with the help of Dr. Ernst Oberortner and the Eugene language (http://eugenecad.org/). Raven assembly design was obtained using the tool web-tool Raven (http://ravencad.org/) Also thanks so Dr. Traci Haddock, Dr. Swapnil Bhata, Evan Appleton, Jenhan Tao and the 2012 BU iGEM team.