Fuse, or Die: The Case for the MoClo Revolution

Boston University iGEM 2013

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Example Construction
Our Solutions

**DESIGNING**
- To use existing CAD tools and design new ones to help define our devices and store our data
- To expand the MoClo library of parts started by the 2012 BU iGEM team and spread the word of MoClo to iGEM 2013

**BUILDING**
- To develop a more comprehensive characterization workflow for genetic circuits containing fluorescent proteins

**TESTING**
- To build a web tool that will produce a standard data sheet to help researchers share their results

**SHARING**
- CAD Tools for Syn Bio
- Updated MoClo Kit
- Characterized Devices
- Datasheet Tool
CAD Tools for Syn Bio
Clotho Apps & Eugene

We have been utilizing Clotho, a synthetic biology software suite developed with tools for analyzing and organizing data.

Bilitchenko et al., (2011) PloS One
Bhatia et al., (2013) ACS Synthetic Biology
**Eugene**

**Design Space**

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**Rules/Constraints**

Device Inverter01(InduciblePromoter, RBS, Repressor, Device Inverter02(RepressiblePromoter, RBS, Reporter) Device Inverter = Inverter01 + Inverter02; println(Inverter);

**Eugene** is a design language for synthetic biology that defines devices, part types, parts, and properties, and constrains their composition.

**Rule-Compliant Device Specifications**

molecule

GFP

RFP
Eugene

//pConstitutive Level 1 Circuits

Property name(txt);
Property sequence(txt);
Property description(txt);

//Promoter
PartType promoter(name, sequence, description);
promoter J23100_AB("J23100_AB", "GGAGTTGACGCTAGCTAGTCTCCT...", "J23100_AB");
promoter J23101_AB("J23101_AB", "GGAGTTTACAGCTAGCTAGTCTCCT...", "J23101_AB");
promoter J23102_AB("J23102_AB", "GGAGTTGACGCTAGCTAGTCTCCT...", "J23102_AB");
promoter J23103_AB("J23103_AB", "GGAGCTGATAGCTAGCTAGTCTCCT...", "J23103_AB");

//RBS
PartType RBS(name, sequence, description);
RBS B0034m1_BC("B0034m1_BC", "TACTAGAGAAGAGGAATACTAAATG", "B0034m1_BC");
RBS B0034_BC("B0034_BC", "TACTAAGAGGAGAAAAATG", "B0034_BC");
RBS BCD1_BC("BCD1_BC", "TACTGGGCCCAGGTT...", "BCD1_BC");
RBS BCD2_BC("BCD2_BC", "TACTGGGCCCAGGTT...", "BCD2_BC");
RBS BCD8_BC("BCD8_BC", "TACTGGGCCCAGGTT...", "BCD8_BC");

//Reporter
PartType CDS(name, sequence, description);
CDS RFP_CD("RFP_CD", "RATGATG...", "E1010m_CD");

//Terminator
PartType terminator(name, sequence, description);
terminator B0015_DE("B0015_DE", "AGGTCCA...", "B0015_DE");

Device pConstitLib(promoter, RBS, CDS, terminator);

Device[] pConstitLibList=product(pConstitLib);
println(pConstitLibList.size());
//save(pConstitLibList);

for(num x=0; x<pConstitLibList.size()-1;x++)
{
    println(pConstitLibList[x]);
}
MoClo vs. BioBricks
MoClo Level 0 to Level 1

Adapted from Weber et al., (2011) *PLoS One*

Level 0 MoClo Parts

Level 1 Destination Vector

Bsal MoClo Reaction

Level 1 MoClo Part
Advantages of MoClo

- One-pot reaction where digestion and ligation occur together
- Advantages:
  - Up to 6 DNA parts together in one step
  - Highly modular with shorter scars
  - Two restriction enzymes and T4 DNA ligase
  - Time and cost reduction

Efficiency of MoClo

MoClo Kit

- 51 Promoters
  - 32 new (dark)

- 16 5’ UTR
  - 12 new (dark)

- 19 CDS
  - 16 new (dark)

- 4 Terminators
  - 3 new (dark)

- 10 DVL0
  - 7 new (dark)

- 4 DVL1
  - (all new)

- 4 DVL2
  - (all new)
Characterized Devices
pConstitutive Library

We have built a total of 78 transcriptional units that constitutively express RFP with different combination of promoters and RBS.
Test combinations of Promoter/RBS to develop heat map and suggest a set to future teams
Flow Cytometry
pConstitutive Library
Controls Required for MEFL Conversion

1. Streak E. coli controls (+ and -) and experimental samples
2. LB agar plates with antibiotics when appropriate
3. Inoculate cultures in triplicate
4. LB supplemented with antibiotics and small molecules when appropriate
5. Dilute overnight culture 1:200 in 1XPBS
6. Run samples through flow cytometer

Spherotech 8-peak rainbow particles (RCP-30-5A) will excite under any wavelength of light between 365 to 650nm. These particles allow for long term performance tracking of the flow cytometer. These are used to obtain the MEFL units for the FITC channel.

Fluorescence Positive Controls - Consitutive Expression of Every Color to be Used

- FITC channel fluorescent protein
- Non-FITC channel fluorescent protein

Fluorescence Positive Controls - Sets of Dual Constitutively Expressed Colors to be Used

- Dual expression of fluorescent proteins

Negative Control

Cells containing no plasmid (or non-expressing plasmid)
Enter data

If you have a form error when you "Submit" this page you may need to reselect your files. Avoid this, click the "Upload" button as you select your files.

**Experiment Name**

Name your experiment.

**Notes**

Any notes about your experiment.

**Device name**

A short name for the device.

**Construct**
Characterizing: 8 Peak Beads

- Run beads every time
  - Highly stable
  - Standard deviation between batches is low
- Beads contain fluorescein
- Calibrate fluorescence in quantitative units: MEFL (molecules of equivalent fluorescein)
Characterizing: Controls

Fluorescence Positive Controls - Constitutive Expression of Every Color to be Used

- FITC channel fluorescent protein
- Non-FITC channel fluorescent protein

Fluorescence Positive Controls - Sets of Dual Constitutively Expressed Colors to be Used

- Dual expression of fluorescent proteins

Negative Control

- Cells containing no plasmid (or non-expressing plasmid)
Fluorescence Positive Controls - Sets of Dual Constitutively Expressed Colors to be Used

Dual expression of fluorescent proteins
pConstitutive Library

Log of RFP (MEFLs) by Bin

Log of Cell Count per Bin
pConstitutive Library

Log of Cell Count per Bin (gray)
Log of RFP (MEFLs) by Bin (purple)

pJ114m1R  pJ124m1R  pJ134m1R  pJ144m1R

We created a web-based app that can generate a datasheet from user input and the Parts Registry.
With the Purdue Biomakers, we fine tuned the features of 2012 datasheet design.
Welcome to the BU Datasheet Generator
Outreach & Human Practices

- We hosted a member of the Marlborough High School iGEM team to get first hand experience on what it is like to work in a synthetic biology lab

- We are writing an article to bridge the gap between synthetic biology and society
Second Annual NEGEM

We would like to thank the Brown-Stanford, MIT, and Wellesley teams and iGEM HQ for participating in NEGEM 2013!
Contribution to iGEM

**Designing**
- Created a Clotho database and Eugene files for our library; worked with the Wellesley iGEM team to develop Eugenie

**Building**
- Expanded the 2012 MoClo kit to 90 Level 0 Parts and 18 destination vectors by adding 63 new Level 0 Parts and 15 new DVs

**Testing**
- Developed a protocol for flow cytometry E. coli experiments and worked with BBN Technologies to improve our data analysis

**Sharing**
- Created a standardized datasheet with the Purdue iGEM Team and an implementation of the datasheet generator web tool is in progress
Future Work

**Future Work**

**DESIGNING**
- Creating complex Level 2 devices and generating scoring system for promoter and 5’UTR strength
- Using Eugenie and Clotho 3.0; populating the new BU ICE Registry from Clotho 3.0

**BUILDING**
- Sharing our flow cytometry protocol with other teams for feedback; continue work on data analysis with BBN Technologies

**TESTING**
- Launching the datasheet web tool and connecting it to Clotho 3.0; create a more compact MoClo Kit based on data analysis

**SHARING**
- Updated MoClo Kit
- Characterized Devices
- Datasheet Tool
- CAD Tools for Syn Bio
Acknowledgments

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Questions?

FUSE, or DIE.
MoClo vs. Other Methods

- Golden Gate and Gibson allow for potentially scar-less assembly without the need for hierarchy
- BUT they require significant planning in oligo design and are not as modular.

http://openwetware.org/wiki/CH391L/S13/DnaAssembly#Molecular_Cloning
Introducing a quorum sensing system to synthetic biology via MoClo

Cloning and characterizing CviR/I and pVioA, a promoter with CviR binding site, into E. coli
pRepressible Library

- Used to generate transfer curve for repressible promoter
- Constitutively expressed RFP correlates to expression of repressor
Grow separate cultures and add varying amounts of arabinose to generate a transfer curve
Inverters
Inverters

[Diagram showing a network of genes and their interactions with arabinose, with log MEFLs (RFP and GFP) on the y-axis and arabinose concentration (mM) on the x-axis.]