The Design, Assembly, and Characterization of a New Library of Standardized Modular DNA Parts

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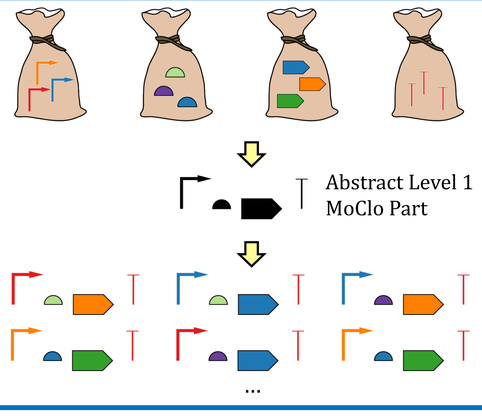
INTRODUCTION

Modular cloning (or MoClo) was introduced in 2011 as a modular, multi-way assembly technique that's based on the Type IIS restriction enzyme strategy used in Golden Gate assembly (Weber et al., 2011). Due to its modularity, ease of use, and low cost, this method is ideal for the creation of a new library of standardized parts for use in synthetic biology.



Here, we present a MoClo library of standardized DNA Parts that includes various promoters, ribosomal binding sites, coding sequences, and transcriptional terminators. The majority of these parts has been converted from BioBricks and will be made available for use through the Registry of Standard Biological Parts.

In addition to the biological experiments, we demonstrate how the wet lab knowledge of MoClo is captured in the synthetic biology software tools that have been developed in our dry lab. These include the Clotho Apps Eugene Scripter and Raven, as well as the new Boston University Center of Synthetic Biology (CoSBi) ICE Registry of Parts.

METHODS

There are three Levels of MoClo Parts in our Library:

- Level 0: Basic part (ex: promoter, RBS, CDS, etc.)
- Level 1: Transcriptional unit (up to 6 L0 Parts)
- Level 2: Composite of up to 6 L1 parts

Basic MoClo (BMC) Reaction: The following components were added to a 0.2 mL tube: 20-40fmol of each DNA component, 10 U of BsaI or BbsI (BsaI for Level 1, BbsI for Level 0 and Level 2; NEB), 10 U high concentration T4 DNA ligase (C#M1794, Promega, Madison, WI, USA), 1 X T4 DNA Ligase Buffer (Promega), and sterile, deionized water to 20 µL. Reactions were performed using the following parameters: 15-25 cycles (37°C 1.5 min., 16°C 3 min.), followed by 50°C for 5 minutes and 80°C for 10 minutes and then held at 4°C until transformed and screened.

Characterization of Transcriptional Units: The RFP expression devices (Table 1) were characterized using a BD Fortessa SORP flow cytometer using a 561nm laser with a PE-Texas Red 610/20 filter. All samples were run in duplicate and data is displayed as a geometric mean with standard deviation error bars.

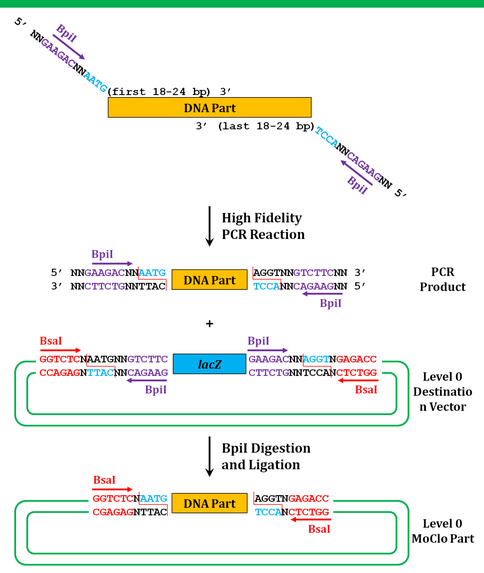


Figure 1: Construction of a Level 0 MoClo Part. Primers are designed for each desired part with the proper flanking sequences shown. High fidelity PCR reactions are done to amplify each part and the PCR product is then cloned into its destination vector using the BMC protocol.

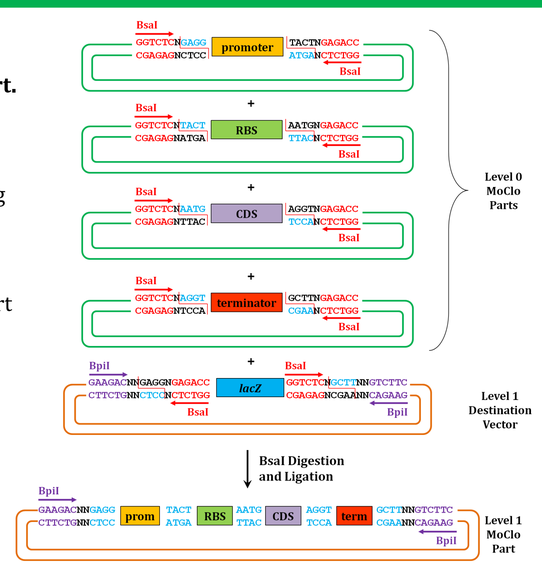


Figure 2: Construction of a Level 1 MoClo Part. Four basic Level 0 DNA Parts (promoter, RBS, CDS, and terminator) have two 4bp non-palindromic fusion sites flanking the DNA Part. By matching fusion sites between the 3' end of the first part and the 5' end of the second part and so on, this enables the proper assembly order of promoter-RBS-CDS-terminator to be maintained.

MOCLO LIBRARY RESULTS

CIDAR Library of MoClo Parts

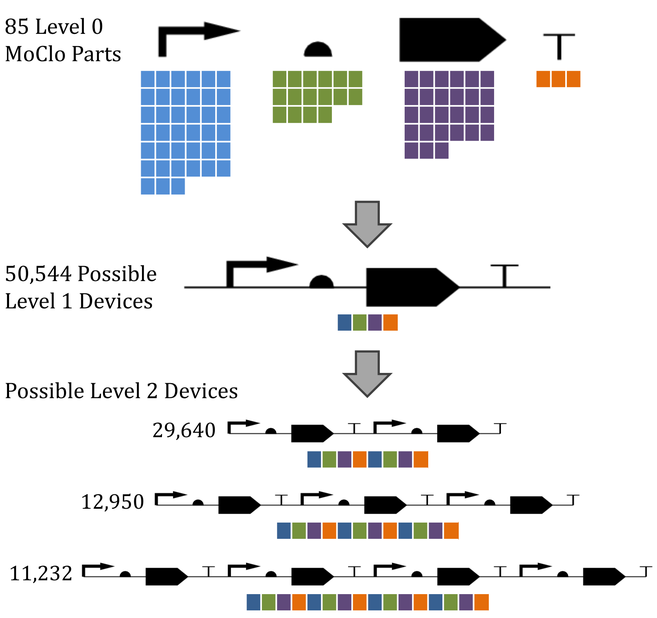


Figure 3: CIDAR MoClo Library. The number of Level 0 Parts we currently have available, with the number of all possible Level 1 and Level 2 combinations that can be made from this library. Level 0 parts shown as colored blocks: promoters (blue), ribosomal binding sites (green), coding sequences (purple) and transcriptional terminators (orange).

Genetic Parts and Devices images made with Pigeon (Bhatia and Densmore, 2013; http://pigeoncad.org/).

Transcriptional Units for RFP Expression

Device				
J004R	J23100	B0034	E1010	B0015
J024R	J23102			
J034R	J23103			
J044R	J23104			
J054R	J23105			
J064R	J23106			
J074R	J23107			
J084R	J23108			
J094R	J23109			
J104R	J23110			
J124R	J23112			
J144R	J23114			
J154R	J23115			

Figure 4: Genetic Devices for RFP Expression. Part numbers refer to DNA sequences obtained from BioBricks parts of the same number (BBa_PART). Promoters are from the Anderson library of constitutive promoters, B0034 is a strong RBS, E1010 is RFP, and B0015 is a strong double terminator.

RFP Expression Using Different Constitutive Promoters

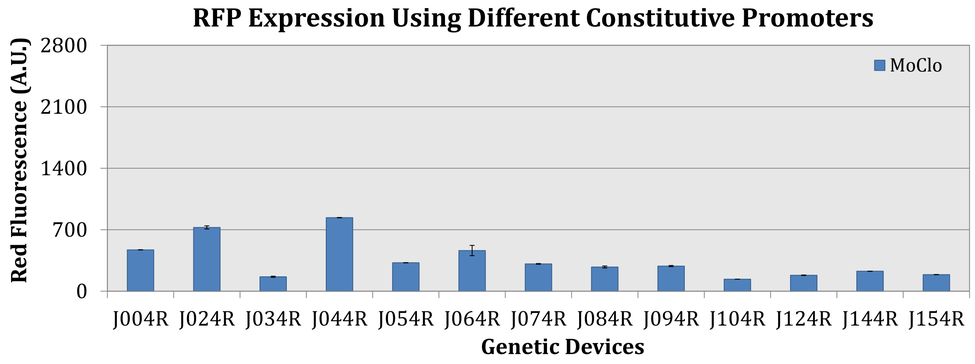


Figure 5: Characterization of Level 1 MoClo Parts. All transcriptional units were made using our Basic MoClo protocol and are in kanamycin resistant vectors.

MoClo vs BioBricks RFP Expression

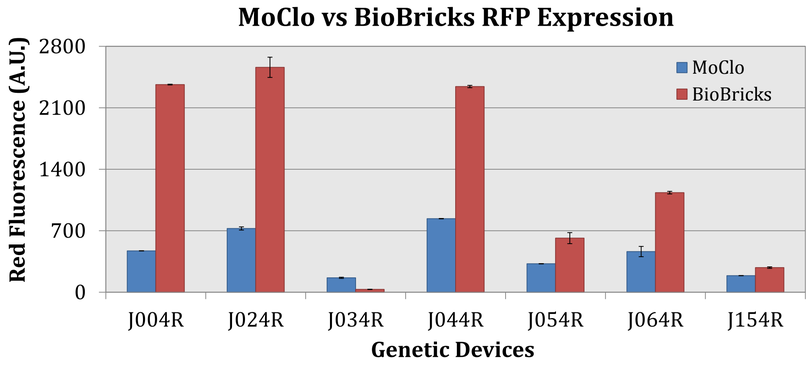


Figure 6: Comparison of MoClo and BioBricks Transcriptional Units. Genetic circuits were made with the same Part sequences following either our Basic MoClo (blue bars; kanamycin vector) or BioBricks (red bars; ampicillin vector) assembly.

MoClo RFP Expression in Three Different Vectors

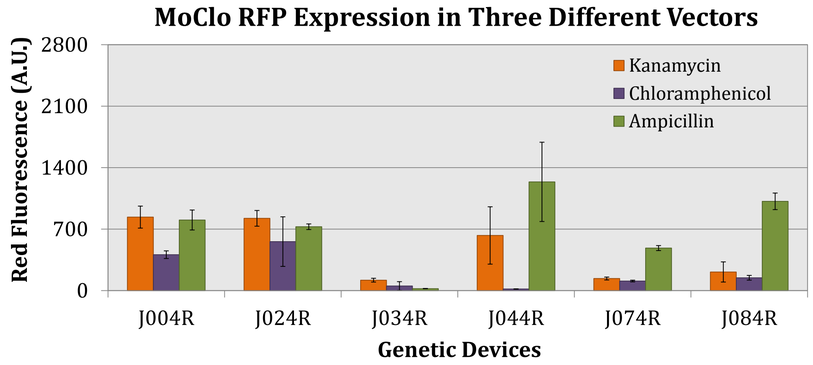


Figure 7: Comparison of MoClo Transcriptional Units in Three Vectors. Genetic circuits were made following our BMC protocol (orange bars, kanamycin) and then digested and ligated into two other antibiotic-resistance vectors (purple bars, chloramphenicol; green bars, ampicillin).

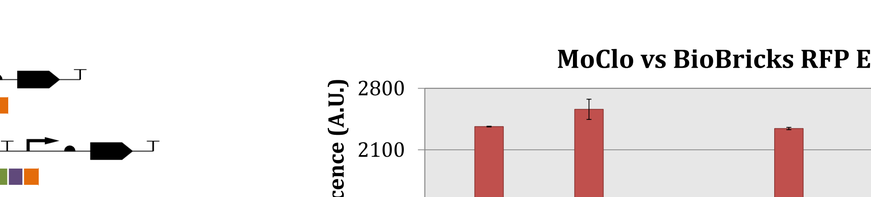
SOFTWARE RESULTS

Eugene Script for All RFP Devices


```
//promoters

Property sequence(txt);
Part promoter(sequence);
promoter J23100_AB("GGAGTTG...");
promoter J23101_AB("GGAGTTT...");
...

//RBS
Part RBS(sequence);
RBS B0034_BC("TACTAGAGAAAGA...");

//Reporter
Part CDS(sequence);
CDS E1010_CD("AATGATGGCTTCC...");

//Terminator
Part terminator(sequence);
terminator B0015_DE("AGGTCC...");

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

Device[]
pConstLibList=product(pConstLib);

println(pConstLibList.size());

save(pConstLibList);
```

Figure 8: Eugene Scripts for Generating the RFP Expression Devices. Properties, part types, specific parts, and devices are defined using Eugene and run using Eugene Scripter in Clotho.

Raven Assembly Graph for Two RFP Devices



Assembly Statistics	Graph structure verified!
Number of Goal Parts	2
Number of Assembly Steps	2
Number of Assembly Stages	1
Number of Reactions	0
Number of Recommended Parts	0
Number of Discouraged Parts	0
Assembly Efficiency	1.0
Parts Shared	0
Algorithm Runtime	153ms

Figure 9: Raven Graphs and Statistics for MoClo Assembly of Two RFP Devices. Raven was used to generate a MoClo assembly graph for two of the RFP expression devices (above). Assembly Statistics are also given as an output from Raven, showing the algorithm runtime and efficiency of the proposed assembly graph , among other statistics (left).

CoSBi ICE Registry of Parts

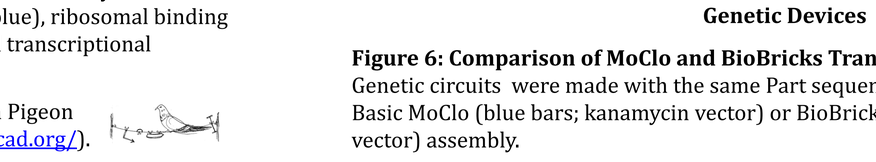


Figure 10: Screenshot of CoSBi ICE Registry of Parts. Currently the ICE registry is set to private as we build our library. This will become open to the public in the future.

DISCUSSION AND FUTURE WORK

Our MoClo Library currently has 85 Level 0 and over 30 Level 1 Parts

- The library is currently private but will be made public on the CoSBi ICE Registry once complete characterization data has been collected
  - Future work will focus on expanding the Level 0 Parts and building and characterizing more Level 1 and 2 Parts

RFP expression is lower but pattern of expression remains the same when MoClo devices are compared to their BioBricks counterparts

- Four silent mutations were introduced to the MoClo RFP to remove illegal cut sites
  - Future work will focus on testing the mutated RFP with BioBricks assembly to continue troubleshooting this problem

Eugene and Raven help speed up the design and build stages done by wet lab users and decrease human error during these stages

- Future work will focus on using these tools for larger, more complex devices for characterizing repressible and inducible promoters and digital logic gates (NOT, NOR, etc.)

Acknowledgments

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