Efficient Modular, Mulitpart and Multiplex DNA Assembly System

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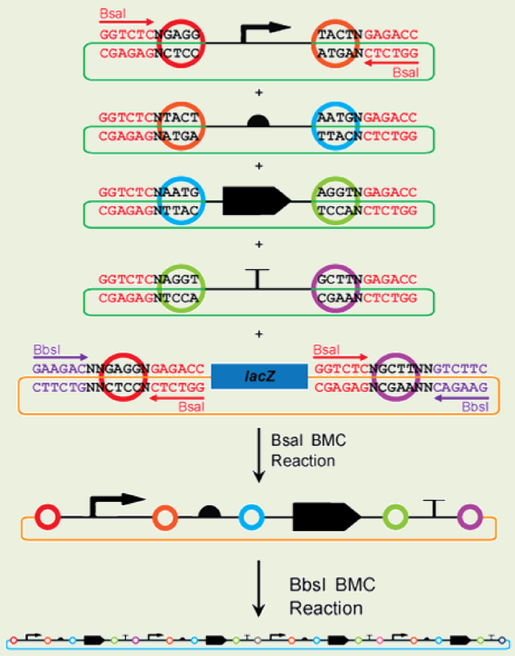
Abstract



MoClo is a one-pot multipart assembly system which uses Type IIS restriction enzymes (BbsI and BsaI) and was published by Weber et al., in 2011. Each of these recognize a 6 bp non-palindromic sequence and cut DNA at a specified distance from that recognition sequence resulting in overhanging 4bp fusion sites. These fusion sites are designed so that multiple parts containing compatible overhangs can be ligated in the correct order with high efficiency. Performed in a single reaction, we can reliably assemble six parts into a single destination vector.

We have improved upon the original protocols and provided a kit of characterized DNA parts and vectors for combinatorial assembly in E. coli. Additionally, we have developed and tested a simple multiplex assembly method with applications for iterative design methodologies, mutant library screens, and expression tuning purposes.

Figure 1: MoClo assembly is based on alternating vectors and antibiotic selection using Type II-S enzymes to dictate fusion sites. Plasmids carrying each part are combined along with a destination vector, all in equimolar concentrations.

Protocol Improvements

- **Weber Protocol**
  - Time: 5 hours
  - Expense: $10 / reaction
    - Volume: 30 uL
    - Enzymes: 20 Units
    - DNA: 40-60 fmol
    - Transformation: 50 uL cells
  - Efficiency: >95% Correct clones (>100 CFU)
  - Library: Non-existent
- **CIDAR Protocol**
  - Time: 45 minutes – 2 hours
  - Expense: $ 1.5 / reaction
    - Volume: 10 uL
    - Enzymes: 5 Units
    - DNA: 10 fmol
    - Transformation: 7 uL cells
  - Efficiency: >95% Correct clones (>100 CFU)
  - Library: 96 part kit, >200 basic parts created

References & Acknowledgements

Manuscript in preparation

Weber et al., *A Modular Cloning System for Standardized Assembly of Multigene Constructs. PloS One.* 2011

Mutalik et al., *Precise and reliable gene expression via standard transcription and translation initiation elements. Nature Methods 2013*

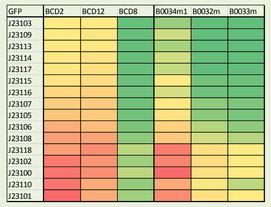
***Acknowledgements**: Jake Beal for assistance with flow cytometry data analysis, 2012 and 2013 BostonU iGEM teams for assistance in building the library.*

For more information, please visit cidarlab.org.

Design and Characterization of CIDAR MoClo Kit

Most of the parts used in the CIDAR MoClo kit have been adapted from the BioBricks Registry (parts.igem.org). Many of these have been slightly modified to adjust for spacing or fix illegal restriction sites. Modified parts are indicated with a lower-case “m’ at the end of the part number. Initially, all J23 series of Anderson promoters (BBa_J23100-J23119) were cloned as _AB promoter parts along with the five Weiss RBSs (BBa_B0030-B0034) and six Bi-Cistronic Design parts (BCDs, biofab.org) cloned as _BC parts to make initial determinations as to promoter and RBS strength by assembling with GFP (BBa_E0040) and a standard terminator (BBa_B0015). These constructs were built using a standard destination vector with kanamycin resistance (DVLK, derived from pSB1K3, pMB1 origin). Sixteen of the J23 promoters, 3 Weiss RBSs and 3 BCDs were used for further characterization. Based on the data in Figure 2, we chose six J23 promoters for the CIDAR kit and proceeded to evaluate expression of these 6 promoters and 6 RBSs in other contexts.

MoClo is based on a system of modular parts and vectors. In order to accurately predict expression we are taking into account the vector, fusion sites, gene order, and gene sequence.



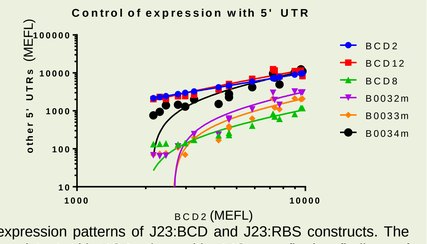


Figure 2: (left) Heat map showing GFP expression patterns of J23:BCD and J23:RBS constructs. The effect of promoter on expression more consistent with BCDs than with RBSs, confirming findings of Mutalik et al. 2013. (right) Expression of each RBS/BCD series of GFP expression cassette sorted by J23 promoter shows reliable behavior of promoters. Weiss RBSs B0032m, B0033m show overall lower expression compared to BCDs. Expression is shown in Mean Equivalent Fluoresceins (MEFLs).

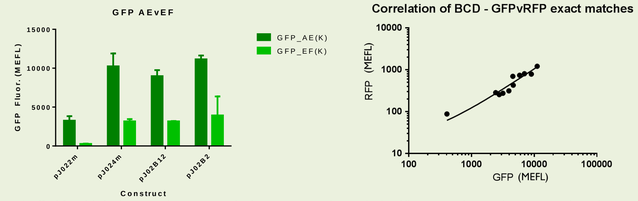


Figure 3: (left) Initial observations revealed a systematic and significant variance in expression due to the fusion sites, presumably the site immediately 5’ of the promoter. (right) Relative strength of expression is not significantly influenced by the coding sequence when BCDs are used in place of RBSs.

pJXXGm_AE series (GFP)
16 promoters x 6 5'UTRS = 96 plasmids in Kan_AE context

pJXXRm_AE series (RFP)
3 promoters x 6 5'UTRS = 18 plasmids in Kan_AE context

pJXXRm_EF series (RFP)
6 promoters x 6 5'UTRS = 36 plasmids in Kan_EF context

Other Contexts
Amp_AE and Amp_EF sets – 18 plasmids (GFP and RFP)
Single color vs two-color sets – 48 plasmids (GFP and RFP)
Blue Fluorescent Protein – 5 plasmids (BFP)

The vectors used are derived from BioBrick standards, pSB1A2 and pSB1K3. Both use a pMB1 origin and are slightly over 2 kb. The following blocks describe the various constructs currently being evaluated.

A kit of 96 parts and vectors is being made available through Addgene to provide a starter system for combinatorial assembly and allow for rapid gene expression tuning. In addition to the parts previously mentioned, the kit will include BFP, Cre, and standard transcriptional regulators (tetR, lacI, etc.) and their appropriate promoters as well as all necessary cloning vectors. We expect this kit to be most useful for traditional biology research groups interested in rapidly developing improved control of gene expression in biological systems for research purposes. Additionally, the kit is intended for use as an educational resource and sample protocols to build simple circuits will be made available at cidarlab.org.

Pigeon (pigeoncad.org) is a webtool for quickly and simply creating SBOL compliant graphics for SynBio designs. pigeoncad.org

Bhatia, Densmore. *ACS Synth. Biol.*, 2013