Multiplex Modular Assembly and Protein Engineering

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Multiplex Modular Assembly

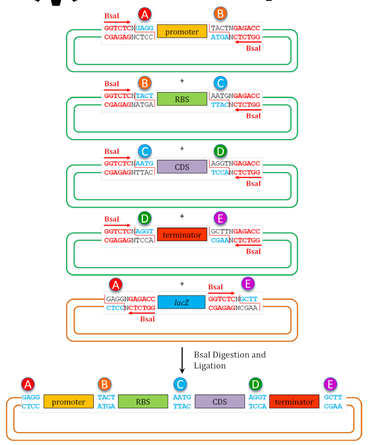


Figure 1: Construction of a Level 1 Transcriptional Unit (TU) by combining four Level 0 plasmids (CAM') with the appropriate Level 1 destination vector (KAN') in a one-pot reaction. While diagrams here show mostly 4 part reactions, at least six parts can be combined with high efficiency.

Modular Cloning, or MoClo, is an assembly technique which utilizes restriction Type IIS enzymes to assemble multiple Parts in a one-pot digestion-ligation reaction greatly decreasing the time and cost of constructing large devices (Weber, Engler et al. 2011). Type IIS enzymes cleave DNA at a specific distance from a non-palindromic recognition sequence. MoClo, Golden Gate (Engler, Gruetzner et al. 2009, Werner, Engler et al. 2012), and related assembly methods take advantage of these enzymes to allow for multipart assembly by designating specific 4-bp overhangs at the cut site for these enzymes.

We have transformed key Parts from the standard BioBricks library into MoClo parts in order to facilitate more efficient and reliable construction of complex devices. MoClo utilizes a multi-level antibiotic based selection system to create large devices. As such, standard pUC19 derived vectors were converted to the appropriate 'destination vectors' for the library by addition of a LacZ reporter flanked by either BsaI (Level 1) or BbsI (Level 0 and Level 2) recognition sequences and the appropriate fusion sites as shown in Figure 1**.**

The Cidar MoClo library currently contains

- 37 promoters
- 13 ribosomal binding sites
- 23 coding sequences (8 fluorescent proteins)
- 4 terminators
- 27 destination vectors.

With variations of this cloning method, we are developing a suite of multiplex modular assembly methodologies which rapidly increase the efficiency of characterization efforts and construction of complex Devices. Multiplex MoClo provides the ability to construct multiple Devices by adding a library of any Part type or transcriptional unit with no significant decrease in efficiency. By creating a library of level 1 parts, it is possible to tune each transcriptional unit by selecting the variant clone which performs as desired via FACS or other analysis.

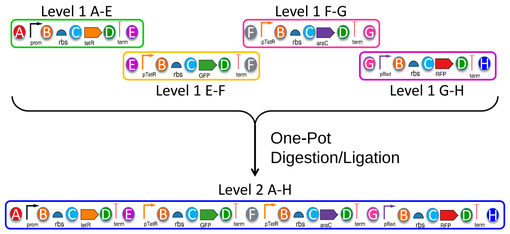
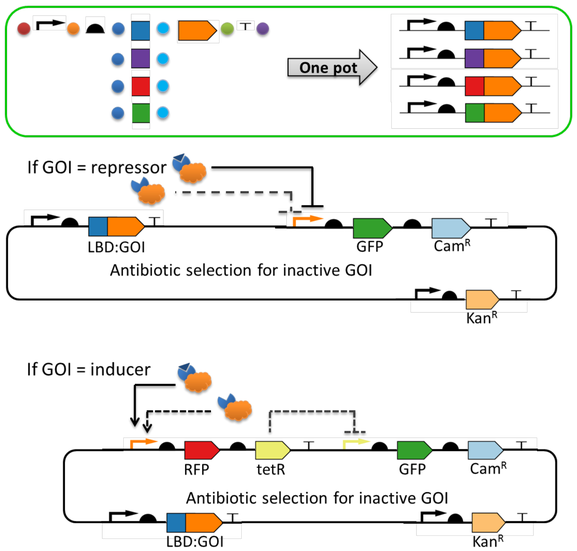


Figure 2: Up to six Level 1 TUs can be combined to create a Level 2 device in the same manner.

Standard MoClo		
		• Single transcriptional unit
Multiplex MoClo (1 part)		
		• Multiple transcriptional units • Screen by size, color, function • Use TU in a combinatorial library
Multiplex MoClo (multipart)		
		• Multiple expression levels with variable Promoter/RBS • Screen by function, protein level • Use to tune complex device - Combinatorial library - Analyze many variants at once - Isolate promising configurations

Engineered Allosteric Control



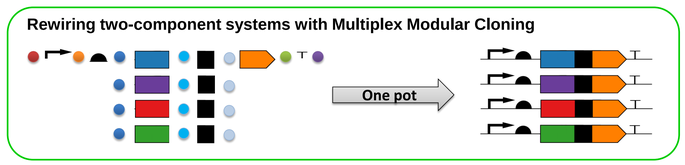
Building on the basic multiplex MoClo method, we are developing a high throughput domain exchange protein engineering assembly and screening system wherein a variety of design candidates are assembled and functionally screened for activity. By attaching ligand binding domains (LBD) to the coding sequence of a particular gene of interest (GOI) in a library-based assembly, we can test hundreds of possible fusion proteins in a single reaction and screen for functional clones to rapidly engineer allosteric control of a given enzyme.

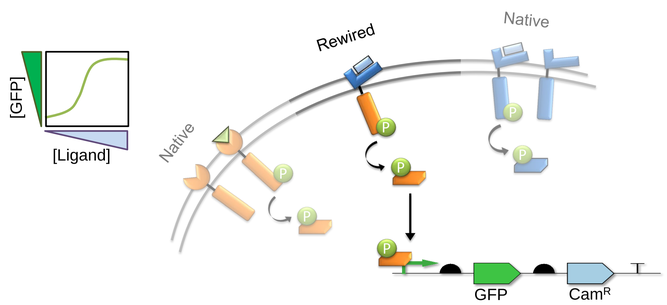
Modular Domain Exchange Protein Engineering

- Decreased time and cost from one-off engineering methods
- Single MoClo reaction creates all iterations
- Screen designed to select for functional fusions during cloning
- Can multiplex promoter/RBS as well to modulate expression level

*Figure 3: **Multiplex Modular Assembly and Screening of Engineered Allosteric Control:** By constructing fusion proteins by linking a coding sequence from a library of ligand binding domains (LBD) to the coding sequence for a gene of interest (GOI) likely a trans activation domain, and assembling these transcriptional units in combination with a selectable marker, only correctly functioning clones will be isolated.*

Rewiring Signal Transduction





Recent work on developing biosensors through domain exchange methods have utilized the histidine kinase family of two-component systems and focused on the light-oxygen-voltage (LOV) sensor domains. The effector domains of these proteins are often highly varied functioning as kinases, transcription factors, and phosphodiesterases. This modularity has previously been demonstrated in the creation of an *E. coli* that detects blue light and responds producing a pigment by combining the light sensor domain with a transcription factor which controls expression of the pigmentation enzyme (Levskaya, Chevalier et al. 2005). We plan to utilize this characteristic of interchangeable domains to test the ability of our system to create synthetic biosensors in an automated fashion and provide design rules for more complex non-heterologous domain exchange.

*Figure 4: **Rewiring two-component systems with Multiplex Modular Cloning:** Previous research has shown that histidine kinase two-component systems are made up of modular domains. MMC may allow for a more rapid design of rewired systems and provide a means by which to study the biological rules and constraints involved in domain-exchange based protein engineering.*

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Acknowledgements: Dr. Swapnil Bhatia, Swati Carr, Evan Appleton, 2012 iGEM team Monique Freitas and Shawn Jin

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