DIYGEM

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DIY-GEM : Developing the Technical Resources and Cultural Underpinnings to Support the DIY-Bio Movement: A Path towards low cost high through-put gene synthesis:

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Abstract

Synthetic biology research requires more cost effective approaches toward reagents and hardware accessibility. We are developing low-cost alternatives to existing hardware and enzymes in an attempt to expand participation in biological research and development.

Our project expands the accessibility of *Taq* Polymerase by engineering it in a form compatible with BioBrick assembly. This allows use of the over-expressed enzyme from a crude bacterial extract in a PCR reaction at a fraction of the cost of highly purified commercial enzyme. In addition, we have developed inexpensive and easily assembled lab equipment such as a gel electrophoresis apparatus and a PCR thermal cycler.

Enabling researchers to synthesize their own enzymes and having access to inexpensive tools will allow for increased participation among the DIY-bio community, stretch increasingly scarce educational funds, and allow rapid scale up of large scale gene synthesis projects.

DIY-Bio and Synthetic Biology

Our goal in this project was to bring together students, faculty, and members of the Baltimore-Washington DIY-Bio Community in an attempt to develop a community of professional and amateur biologists interested in synthetic biology. To that end we formed the Baltimore-US team with the purpose of participating in the 2010 iGEM competition. During late spring and early summer of 2010 evening meetings were held to introduce and discuss the Bio-Brick assembly standard and the Registry of Standard Biological Parts and weekend workshops were held to introduce basic lab techniques needed by the team once they focused on a specific project.

Because of our association with the DIY-Bio community we also embarked on a discussion of issues facing the DIY-Bio movement including laboratory safety, biosecurity, and resource accessibility. The Baltimore-US team seeks to join the dialogue regarding the involvement of citizen scientists in synthetic biology research. As part of this effort we worked with representatives of federal agencies to understand the biosecurity issues facing synthetic biology and the DIY-Bio movement. Members of our team attended the Presidential summit on Bioethics related to synthetic biology. Other members of our team undertook web based training in biosecurity and good laboratory practices provided by the CDC.

Team Baltimore US: Safety Code

We recognize the importance of each member's personal responsibility to the safety and security of our labs and our work. This includes the duty of not ignoring another's unsafe or possible harmful actions. We are committed to acting in a responsible manner and taking a pro-active approach to staying current with local, national, and international laws, regulations and guidelines. We are dedicated to being informed about principles and practices designed to prevent hostile use of our labs, equipment, materials and products. It is our duty to contemplate the safety and security issues that may arise as an outcome of our projects.

Developing low-cost alternatives to existing enzymes: *Taq* polymerase

One of the issues facing the DIY-Bio movement, but also confronting professional scientists and educators is access to the tools necessary to conduct synthetic biology projects. Central to this is the cost and availability of enzymes such *Taq* polymerase, an essential component of the core technique of PCR, and inexpensive and readily available hardware to analyze the results of these experiments. Curiously, what would seem like obvious parts available through the registry-namely a source for enzymes necessary to build systems from registry parts were absent. Therefore team Baltimore-US set out to explore the possibility of providing a "tool box" that could be used in the assembly of standard parts.

Project Design

Thermus aquaticus, the source organism for *Taq* polymerase was readily available from the laboratory of one of our team advisors and was chosen as our initial target. Poli Coli (our team name for the project) was envisioned to be a convenient and inexpensive source of a key ingredient in synthetic biology manipulations –the thermostable *Taq* polymerase enzyme. The expiration of patent protection has brought the cost of *Taq* polymerase down considerably and many laboratories now make their own "home brew" *Taq* from the cloned gene. However, including the *Thermus aquaticus pol*I gene in the Standard Parts registry is complicated by the presence of restriction enzyme recognition sites (*Pst*I) incompatible with the BioBrick assembly standard. Therefore in order to produce a part consisting of the *Taq* polymerase coding sequence it would first be necessary to eliminate the *Pst*I site without changing the amino acid sequence of the protein.

Poli Coli: Point mutagenesis to remove *Pst* I site from *Taq* polymerase gene:

Plan A. In order to remove the *Pst* I site without changing the amino acid sequence of the *Taq* polymerase protein we used PCR and primers designed to change the sequence CTGCAG to CTTCAG (Figure 1), both CUG and CUU are codons for the amino acid leucine. These mutagenic primers, in conjunction with BioBrick prefix and suffix primers were used to amplify the 5' and 3' ends of the *Taq* polymerase gene from *Thermus aquaticus* genomic DNA (Figure 2). The products of these reactions would then be denatured, annealed, and the 3' ends extended to create the full length gene compatible with the BioBrick assembly standard.

GTGGAGAAGATCCT(T)CAGTACCGGCGG

Mutagenic primer #1

CACCTCTTCTAGGA(A)GTCATGGCCGCC

Mutagenic Primer #2

GTTTCTTCGAATTCGCGGCCGCTTCTAGATGCTGCCCCTCTTTGAGCC

BioBrick + Taq Prefix Primer

GTTTCTTCCTGCAGCGGCCGCTACTAGTATCACTCCTTGGCGGAGAGCC

BioBrick + Taq Suffix Primer

Figure 1. Primers used for the mutagenesis and addition of BioBrick prefix and suffix to the *Taq* polymerase gene.

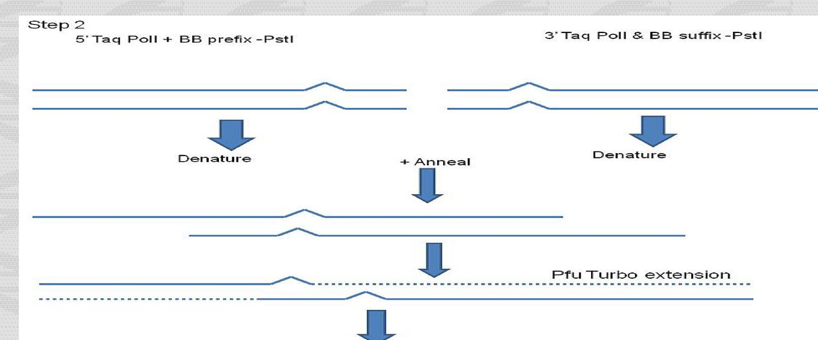

Figure 2. PCR reaction #1 and #2 to create 5' and 3' products. The two products are designed to overlap by 15 nucleotides thus facilitating annealing and later extension.

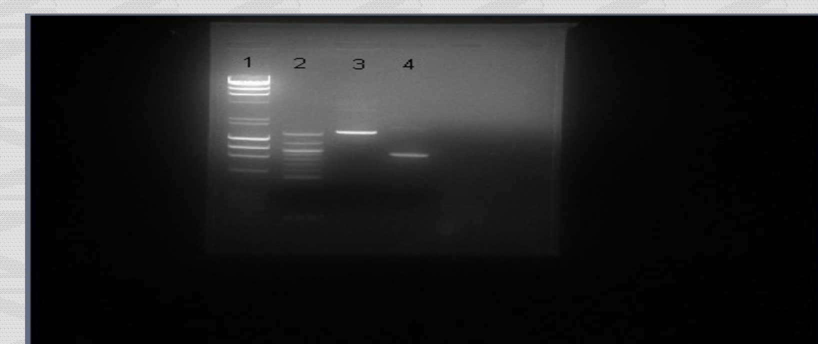

Figure 3. results of PCR amplification of *T. aquaticus* 5' and 3' regions. Lane 1-1Kb ladder, 2-100 bp ladder, 3-PCR using BB prefix and mutagenic primer 1, 4-PCR using BB suffix and mutagenic primer #2. Pcr reactions were performed using *T. aquaticus* YT-1 as template.

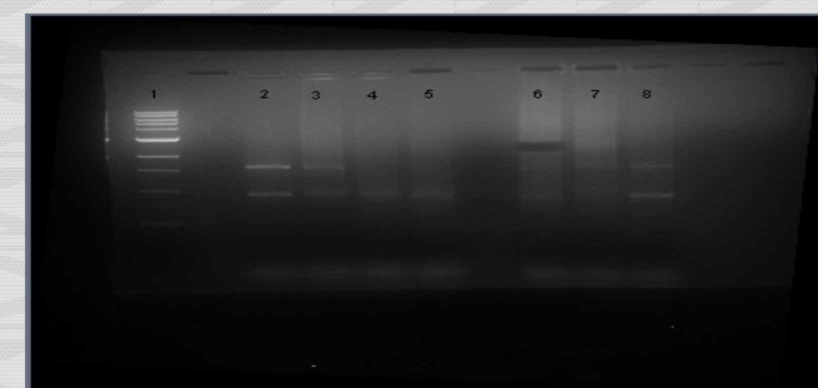

Figure 2. Results of Overlap extension using PCR products from Figure 1. Lane 1-1 Kb ladder, Lane 2– 8 Results of temperature/DMSO gradient for annealing temperature. 2.5 Kb band in lane 6 excised and purified using Quiagen Gel Extraction Kit.

An Alternative Strategy

Unfortunately , after many attempts at the overlap extension and amplifying the results we failed to obtain a PCR product of the expected size for the intact *Taq* polymerase gene. Therefore we elected to pursue an alternative strategy based on the Stratagene Quick Change protocol. In this method, a previously cloned *Taq* polymerase is used as the template in a PCR reaction with the two mutagenic primres (Figure 1). Following amplification, the template is digested with the enzyme *Dpn* I and the PCR product transformed into competent cells. Colonies are then analyzed for the presence of the mutation. This method suffers from the drawback that the BioBrick prefix and suffix must be attached in a subsequent PCR reaction.

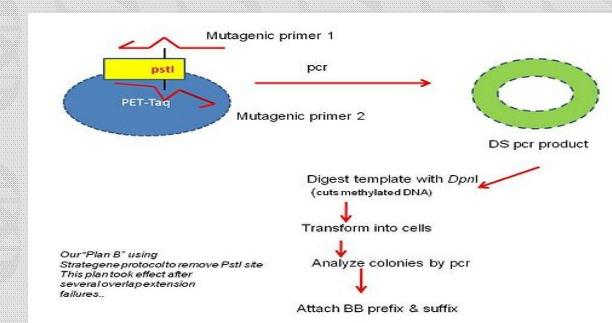

Figure 5. Final strategy (the one that worked) for introducing a single base change in the sequence of the *T. aquaticus pol* I gene and the addition of BioBrick prefix and suffix. Outline of mutagenesis strategy based on Stratagene Quick-Change protocol.

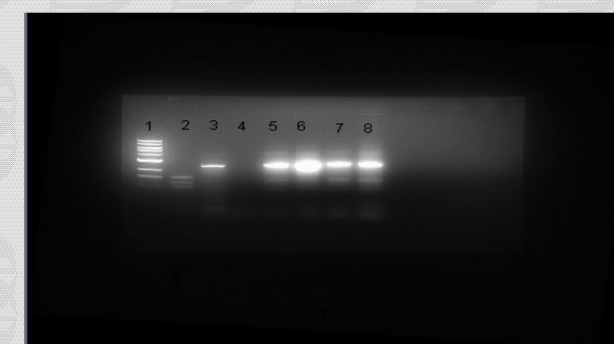

Figure 6. Colony PCR using BB prefix and suffix on colonies following Quick-change mutagenesis, *Dpn*I digestion and transformation into NEB –10α cells. Lanes: 1-1Kb ladder, 2-100 bp ladder, 3– T. aquaticus genomic DNA + control, 4-colony pool A, 5-colony pool B, 6-colony pool C, 7-colony pool D, 8-colony pool E. Bands were excised from the gel, purified using the Quiagen Gel Extraction Kit, digested with *Eco RI*-HF and *Pst* I-HF, gel purified again and ligated into plasmid pSB1C3

Figure 7. Chloramphenicol resistant colonies after ligation of modified *Taq* polymerase gene and pSB1C3. Colonies are currently be analyzed by PCR prior to sequence analysis and submission to the registry.

Developing low-cost alternatives to existing hardware

Before and during the development of Poli Coli we realized that due to the cost and effort required to get biological hardware tools, researchers were unfortunately limited to low throughput processes. To ameliorate this we conceived several plans, such as developing an optimizing a process to increase the throughput of biological parts or methods to make certain common operations more expedient. Eventually we realized that a quick way to increase the expediency of biology would be to perform massive parallel operations simultaneously. However such parallelism requires a considerable investment in hardware that most researcher cannot endure. Since our project theme was to enhance the accessibility of biology and that actual hardware for biological development was either difficult to obtain or prohibitively expensive for the average person, it became clear we needed to develop a cheap alternative to conventional lab hardware.

To this end we developed a cost effective small system for electrophoresis and thermocyling. These two operations we found to be the most onerous and the largest bottle neck that could be reasonably shrunk using parallelism. Additionally these tools are the basis for many, if not most, biological experiments that citizen scientists may want to perform. The platform currently consists of a small power supply, a control panel (Figure 9), and an electrophoresis gel box (Figure 8). Unfortunately due to time constraints the thermocycler was not completed, however the control panel is fully capable of controlling a PCR should we build one in the near future. The control panel is controlled by a simple PC interface and allows users to vary the voltage from 100V to 0V. Additionally it allows the user to specify a ramp voltage and set a timer. The control panel is meant to be a universal controller for a set of biological equipment; though currently it only supports the electrophoresis and PCR, in the future it could support other cheap biological tools.

Figure 8. Horizontal gel electrophoresis box. Cost of this unit is projected to be less than $10.00

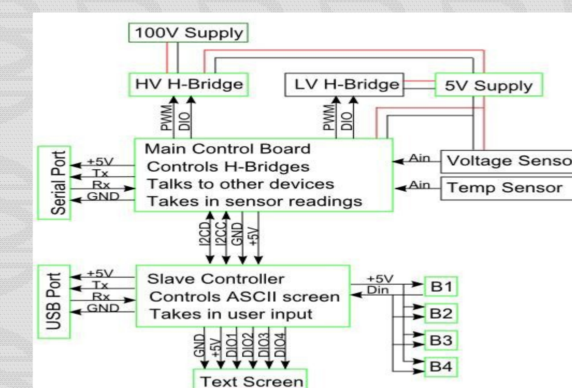

Figure 9. Diagram of proposed "Universal" control that can be used to control and power thermocyclers, electrophoresis units and other lab equipment.

Summary & Conclusion

In forming our iGEM team, Team Baltimore brought together a diverse collection of amateur and professional scientists to explore the field of synthetic biology and to discuss issues surrounding the DIY-Bio movement. We showed that the open access model of local Community Colleges can be used by those interested in the DIY-movement to learn about and gain expertise in the techniques central to the field of synthetic biology and the DIY-Bio movement while at the same time offering the institutional support necessary to alleviate the publics concerns and perceived dangers of DIY-Biology.

In addition, Team Baltimore has contributed to the availability of open, accessible tools necessary to conduct DIY-Bio experiments by reformatting the *T. aquaticus pol*I gene so that it is compatible with the BioBrick assembly standards. Although we did not complete our reformatting in time for submission to the Standard Parts Registry for the 2010 iGEM competition, we anticipate submitting our part in the next few weeks after DNA sequencing confirms our reformatting. Team Baltimore has also successfully developed inexpensive, easily constructed hardware for agarose electrophoresis at a fraction of the cost of commercially made instruments. These tools collectively have the potential to significantly reduce the cost of doing experiments in both professional and amateur laboratories as well as teaching institutions.

Team Baltimore will continue in it's efforts to promote safe and sustainable practices in the DIY-Bio community and to increase access to the tools of synthetic biology. Future experiments will focus on inserting promoters, RBS, and terminators for the efficient expression of the POLI Coli Taq, comparing the POLI Coli Taq to commercial preparations and developing robust protocols for the expression and purification of POLI Coli Taq. In addition we will continue the development of easily accessible hardware tools for exploring the field of synthetic biology.

Acknowledgments:

This work was supported in part by the National Science Foundation Department of Undergraduate Education—Advanced Technology Education Project # 0903208. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation

Team Baltimore wants to thank their sponsors including The Northeast Biomanufacturing Center and Collaborative who supplied registration and travel funds. Axygen Biosciences, New England Biolabs, and Quiagen who supplied laboratory supplies and reagents and The Community College of Baltimore County for providing laboratory and meeting space.