DIY-GEM: Tools for citizen-scientists

Baltimore-US
A little bit about who we are

We are:

▶ Excited by the idea of ubiquitous synthetic biology.
▶ Sympathetic to the DIY ethic.

We want to know:

$$\frac{?}{\text{biology}} = \frac{\text{breadboards}}{\text{electronics}} = \frac{\text{amateur rockets}}{\text{physics}}$$

and how can we help?
What is the potential of DIY science?

Imagine if you could brew polymerase in an afternoon:

- PCR in high schools?
- External wet drives?
- Would you like some genetic sequencing with your birdwatching?
How should we think about this potential?
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Citizen Scientists Attract FBI's WMD Unit

By Michelle Williams

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Looking at safety as a priority, the feds reach out to members of a new movement that takes science into neighborhoods.

- Invited liaisons from the WMD Directorate to speak to our group.
- Attended Presidential Summit on Bioethics
- Participated in 1st FBI-DIYBIO workshop
Our Project: a kit for citizen-scientists

We have developed:

▶ BioBricked Taq polymerase
▶ A gel-electrophoresis kit (some assembly required)

We are in the process of developing:

▶ PCRIY: an at-home thermal cycler.
Taq Polymerase on the cheap

- Polymerase is a necessary ingredient for PCR.
- But it's expensive: on the order of 10 cents / unit of activity.
- And what if you can’t find someone to sell it to you?
How we planned to do it

1. Design Taq primers with BioBrick prefix and suffix.
2. PCR Taq, yielding Taq with BioBrick ‘fixes attached.
3. Cut and ligate into standard assembly vector.
A bit of a problem

\[ \text{\textit{T. aquaticus Pol}} \text{ gene} \]
Why is this a problem?

Cut with *SpeI* and *PstI*
Remedy: overlap extension mutagenesis

**Reaction #1**

[Diagram showing the process of overlap extension mutagenesis with PCR and PstI site disrupted.]
... *Simile* for complement

**PolI gene**

```
5'  3'  5'  3' 
```

**PolI gene**

```
PstI site disrupted
```

**PolI + BB suffix**

```
3'  5'  3'  5' 
```

**GACTCTG**

**PstI site disrupted**

**Reaction #2**

```
5'  3'  5'  3' 
```

**PstI site disrupted**

```
GAACCTG  BB suffix
```

**PolI gene**

```
PstI site
```

**PolI + BB suffix**

```
GACTCTG  BB suffix
```

**3'**

**5'**

**3'**

**5'**
... and combine!

Product from Reaction #1

5' BB prefix Poll CTTGAC 3'
3' ______________________ 5'

Denature, Anneal

5' BB prefix Poll CTTGAC 3'
3' ______________________ 5'

GAAGCTG BB suffix

Extend with Pfu Turbo

5' BB prefix Poll CTTGAC 3'
3' ______________________ 5'

GAAGCTG BB suffix
Results of PCR amplification of *T. aquaticus* 5 and 3 regions

- Lane 1: 1Kb ladder
- Lane 2: 100 bp ladder
- Lane 3: PCR using BB prefix and mutagenic primer #1
- Lane 4: PCR using BB suffix and mutagenic primer #2.

PCR reactions were performed using *T. aquaticus* YT-1 as template.
Results of overlap extension

- Lane 1: 1 Kb ladder,
- Lanes 2-8: Results of temperature/DMSO gradient for annealing temperature.

2.5 Kb band in lane 6 excised and purified using Qiagen Gel Extraction Kit.
Overlap extension disappoints

- Initial rounds PCR yielded products of approximately the correct size.
  - Reaction #1: 1500bp
  - Reaction #2: 1000bp

- Yet we were unable to isolate a correctly-sized product for the overlap (never able to amplify):
  - Expected: ca. 2500bp
  - Consistently yielded 900bp instead.

- This suggested that the binding sites for our PCR primers were not unique.

- With the deadline approaching, we attempted another protocol.
Site-directed Mutagenesis

Note:

- Overlap extension attempted with genomic Taq DNA.
- Quick-change protocol performed with a cloned PolI gene.
Colony PCR using BB 'fixes on colonies following Quik-change mutagenesis

- Lane 1: 1Kb ladder
- Lane 2: 100 bp ladder
- Lane 3: *T. aquaticus* genomic DNA (+) control
- Lane 4: colony pool A
- Lane 5: colony pool B
- Lane 6: colony pool C
- Lane 7: colony pool D
- Lane 8: colony pool E

DpnI digestion and transformation into NEB 10α cells. Bands were excised from the gel, purified using the Qiagen Gel Extraction Kit, digested with *EcoRI*-HF and *Pst I*-HF, gel purified again and ligated into plasmid pSB1C3.
Success!

![Image of petri dish with bacterial colonies labeled "ID 350 µl"]
Future Directions

▶ promoter, RBS, purification tag
▶ validation, assay
▶ purification kit

We look forward to developing:

▶ BioBricked restriction enzymes, ligase
▶ A purification kit for Taq
▶ an oligonucleotide synthesizer.
Hardware

What if we had more economical tools?

- Wider access
- Higher throughput

Our goal was to develop hardware with these specific attributes:

- Inexpensive
- Ease of construction
- Ease of use of the final product
- Replicates common lab equipment
Development of an electrophoresis gel box

We began by developing a tool common to most laboratories. What we wanted:
▶ A box to accommodate a mini-gel
▶ A parallel anode/cathode pair spanning its width
▶ Appropriate safety devices

Additionally we also were looking to develop a thermal cycler. We wanted the following:
▶ Programmable thermal cycling
▶ Capability for at least 8 microfuge tubes
▶ Heated lid
▶ Appropriate safety devices

Overall we wanted both devices to be compatible with the same computer interface.
An inexpensive electrophoresis kit

Our team has developed an assembly kit for an electrophoresis box. It consists of:

- Chassis
- Conducting plates
- Interlock

Several of these kits were built and tested.
An inexpensive electrophoresis continued

Construction of the electrophoresis consists of:

- Consumer-grade parts (Conducting plates, Bananna Plugs)
- Laser-cut parts (Acyrlic)
- Interoperable with standard power supply or our Control Unit.

Pricing:

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See wiki for assembly directions.
Development of the control unit

We decided on designing a unified control unit for our hardware the tools we were designing to prevent a replication of electronics and to decrease overall price. The control unit can be controlled by the onboard interface or via PC.

We built a controller with the following:

- A high power bridge
- A low power bridge
- Four generalized sensor inputs
- Computer interface
- User interface
Future Directions in Hardware

Near-Term:

- Complete the thermal cycler design and test.
- Fix up the unified control unit.
- Polish user interface for control.

Mid-Term: Develop new tools compatible with the unified control unit such as a spectrophotometer, adjustable centerfuge, or an incubator.

Far(far)-Term: Develop more intricate tools such as an oligonucleotide synthesizer.
Acknowledgments

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Thanks and Questions?