SynBio Is Expensive

iGEM is great. Brings synthetic biology to the masses. But it’s really expensive.
- Enzymes, Digestions, Ligations
- Equipment - Thermocycler, Incubator, Centrifuge
  Minipreps, Gels, etc

Want to help other teams as well!

We decided to look at DIY approaches to synthetic biology to make iGEM accessible.
# DIY Cost Comparison

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Safety

Emerging new field of biotechnology - a new hope, a new worry...

Our Project:

- Creating the tools
- Bottleneck to control access to dangerous DNA
The Plan

Recycling Methods:
- Minicolumns
- Gels

DIY Methods:
- Minicolumns
- Enzyme Purification
  - RTX & ELP
  - Gel Purification
- Enzymes
  - EcoRI, M.EcoRI
### DIY Cost Comparison

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Comparing costs between "Ours", "Current", and "Commercial".
DIY - Homemade Minipreps

- DIY Buffers
- Minicolumn Recycling
## DIY Buffers

- **Homemade Buffer Efficiency**

<table>
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<tr>
<th>Buffer Type</th>
<th>Yield</th>
<th>Efficiency</th>
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<tr>
<td>QIAprep Spin Miniprep Kit</td>
<td>219.6 ng/µL</td>
<td>-</td>
</tr>
<tr>
<td>Re-suspension Buffer Only</td>
<td>189.1 ng/µL</td>
<td>86%</td>
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<tr>
<td>Lysis Buffer Only</td>
<td>221.5 ng/µL</td>
<td>101%</td>
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<tr>
<td><strong>Neutralization Buffer Only</strong></td>
<td><strong>102.8 ng/µL</strong></td>
<td><strong>47%</strong></td>
</tr>
<tr>
<td>Binding Wash Only</td>
<td>240.2 ng/µL</td>
<td>109%</td>
</tr>
<tr>
<td>Column Wash Only</td>
<td>239.5 ng/µL</td>
<td>109%</td>
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- **Increased overall efficiency from ~50% to ~60%**

Klenchin (2010).
## Minicolumn Recycling

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<tr>
<td>New Minicolumn</td>
<td>175.8 ng/uL</td>
<td></td>
</tr>
<tr>
<td>Recycled Minicolumn</td>
<td>178.7 ng/uL</td>
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100% Efficiency

0% Carryover

DIY Minicolumns & Gel Recycling
## DIY Enzymes

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### Current Costs

- Transformations: $8.25
- Plating: $0.71
- Overnights: $0.15
- Digests: $0.80
- Ligations: $6.40
- Minipreps: $1.74
- Gels: $2.70

### Comparison

- **Ours**
  - Transformations: $8.25
  - Plating: $0.71
  - Overnights: $0.15
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  - Gels: $2.70

- **Commercial**
  - Transformations: $8.25
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ELP (Elastin Like Protein)

- Oligomeric repeats of Val-Pro-Gly-Xaa-Gly
- Fused at ends of proteins and precipitates at elevated temperatures and increased concentrations of NaCl.

*Meyer (1999)*
RTX (Repeats in Toxin)

- Polypeptide repeats
- Undergoes conformational change upon binding to Calcium which causes them to precipitate.
- We submitted an RTX to the registry in Freiburg format.

BBa_K1188002
RTX Characterization

- Fused RTX to GFP
- Grew in culture
- Lysed cells
- Treated lysed cells with various CaCl

Results show 10mM concentrations of Ca are effective in precipitating tag when attached to GFP
RTX
Gel Purification with Color Tags

- Agarose Gel
- AmilCP and RFP
- Quick
- Easy to do
- High purity
- Decent yields
- Utilizes only common lab materials
Characterization

AmiCP

RFP

Mixed

Absorbance vs. Wavelength (nm) for AmiCP, RFP, and Mixed samples at different concentrations.
Results of Separation

20% Yield

34% Yield
The Plan
Making the Plasmids

- Origin of sequence: REBASE
  - Codon optimized
- Process
  - Gibson Assembly
  - PCR
  - Digestion/ Ligation
Unfortunately...
Summary

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Outreach

High School Curriculum

- HS science labs can range thousands per year
- Key curriculum involves gel electrophoresis, restriction digestion, ect.
- Future iGEMers in peril!

How can DIY bio help?
Outreach (cont.)

The iGEM Experience: Creating DIY, safe, low-cost biology for the mass through kits.

Transformation (Chemical)
- DNA
- Chemically Competent Cells
- Antibiotics

Digestion
- Enzymes: E, P, X (DIY)
- 10x NEBuffer 2.1
- DNA

Gel Electrophoresis
- DNA
- Loading Dye
- DNA Ladder
- Agarose powder
  (Recycle)
- Sybr Safe
Concluding Notes

Excerpt from “Biopunk, DIY Scientists Hack the Software of Life”:

"We reject the popular perception that science is only done in million-dollar University, government, or corporate labs; we assert that the right of freedom of inquiry, to do research and pursue understanding under one's own direction, is as fundamental a right as that of free speech or freedom of religion."
Thanks to Our Sponsors!!
Questions?