Automating Protein Production Using Multi-chromatic Light

McMaster University
Hamilton, Ontario, Canada
Who are we?
How do we improve or build on current biological protein expression systems?
Recombinant Protein Expression
Recombinant Protein Expression
Gene Induction

Gene expression controlled by the addition of an inducer

**Advantages**
- Straightforward
- Well characterized
- Controlled

**Disadvantages**
- Requirement to make or purchase the chemical
- Non-reversible
- Human intervention
- Install expensive automated fluidic systems

How do we make gene induction easier and more efficient?
Light-Inducible Systems

Phytochromes and Cyanobacteriochromes
- Within plants and cyanobacteria like Synechocystis PCC6803
- Tabor et. al (2011) have demonstrated two cases

Transforms external light stimulus into a transcriptional response
Recombinant Protein Expression

All of the designs, constructs, and experiments presented in this poster were performed by the students of the 2015 Hamilton McMaster University team.
Cell Lysis

Mechanical (French press, sonication, freeze-thaw)
Chemical (detergents, alkaline lysis)
Biological (lysozyme)

Exist and work and serve their purpose, but:
• Costly
• Contaminate
• Denature
• Some are not applicable to all cells
• Human intervention

How do we make cell lysis more ubiquitous and effective?
Viral Holin and Endolysin

- Utilized by viruses during replication
- Forms holes in membrane, leading to:
  - Depolarization
  - Release of endolysin
Project Design

All of the designs, constructs, and experiments presented in this poster were performed by the students of the 2015 McMa
ster University team.

Visit Our Wiki
2015.igem.org/Team:Hamilton_McMast
Plasmid Design – Chromophore

- **pSB1A3**: AmpR
- **Backbone**
- **BBa_I0500**: pBad/araC
- **BBa_I15008**: ho1
- **Bba_I5009**: pcya
**Plasmid Design – Red**

**pSB1K3:** KanR Backbone  
**Bba_I14018:** p(Bla)  
**BBa_K592018:** cph8  
**BBa_K769001:** PompC-GFP
Plasmid Design – Green

pSB1C3: CamR Backbone
Bba_I14018: p(Bla)
PCR’s from pJT122: ccaS
PCR’s from pJT122: ccaR
PCR’s from pJT122: PcpcG2
BBa_K112000: T4Holin
Bba_L112806: T4Endolysin
Tabor et al., 2011
Methodologies

Process:
Source DNA
* Obtained from Standard Registry of Biological Parts
** Obtained from Addgene

Green Plasmid:
CamR Backbone
pSB1C3 *
pbla
BBa_I14018 *
Ccas-Ccar
PCR from pJT122 **
Pcpcg2
PCR from pJT122 **
T4 Holin
BBa_K112000 *
T4 Endolysin
BBa_K112806 *

Red Plasmid:
KanR Backbone
pSB1K3 *
pbla
BBa_I14018 *
cph8
BBa_K592018 *
PompC-GFP
BB_K769001 *

Chromophore:
AmpR Backbone
pSB1A3 *
pBad/araC
BBa_10500 *
ho1
BBa_I15008 *
pcya
BBa_15009 *

Culture
Miniprep
Digestion
Gel Extraction
3A Assembly
Transformation
Sequencing

DH5α Competent E. coli
BL21 Competent E. coli
Methodologies

- Induction of Protein Expression
- Cell Lysis
- Protein Harvesting

- 705nm red light induction
- 535nm green light induction
- Proof of Concept via GFP
Results – $p(bad)$-ho1 (S1PC)
Results – $p(bad)$-ho1

Successful transformation of completed gene section

Sequence verification

$pBab/araC$ → $ho1$ → $AmpR$ → $Chromophore System$ 3732 bp → $pcya$
Results – Enzymes & Buffers

Digestions stopped working: Parallel tests to resolve

<table>
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<th>C.S. 3.1</th>
<th>C.S. 3.1</th>
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<tr>
<td>E&amp;S</td>
<td>E&amp;S</td>
<td>E&amp;P</td>
<td>E&amp;P</td>
<td>X&amp;S</td>
<td>X&amp;S</td>
</tr>
</tbody>
</table>

CutSmart worked

NEB 3.1 did not work
Results – PCR with overhang

• Parts required for light sensitivity
• Not available on campus
• 3 genes were PCR’d out of a cassette (Addgene):
  \( P_{pcpg2} (~300 \text{ bp}) \)
  \( ccaS (~2'000 \text{ bp}) \)
  \( ccaR (~700 \text{ bp}) \)
• Overhangs of E, X, S, and P, cut sites were introduced using primer design

Pending submission to iGEM as new BioBrick
Results – Progress Report

Progress
• Constructed majority of Chromophore plasmid
• Learned, invested into team education
• PCR’d parts with BioBrick compatibility

Resolved Difficulties
• Starting late in the year ✓
• Digestions ✓
Hardware

Simple by design – prototype developed
Hardware

1. Box encloses shaker within incubator
2. Far red light (705nm) induces POI production
Hardware

3. Green light (535nm) to lyse cells
Human Practices – Outreach

Social Media (Facebook, Twitter) and website
Week 5: Digestion (not the eating kind!)

Surprised? I’m back again, two weeks in a row! Usually this would be Max’s post, but he’s a bit busy this week, so... this is my opportunity to enjoy your support. Today, we’re going to talk about the ‘classical’ method that you have probably learned about in high school. If you missed last week’s post on Miniprep, read that first.

The restriction digest, commonly known as digestion, is the process in which a DNA strand or plasmid is cut at specific DNA sequences, called restriction sites. This happens through hydrolysis, which is probably why the name of the process is digestion - the backbone is “digested” at the restriction site into smaller pieces. We use restriction enzymes, which recognize the specific restriction sites on DNA and cut at that location. Restriction enzymes are associated with bacteria, as a defense mechanism for destroying viral DNA.

Week 05: Digestion (not the eating kind!)
Collaboration

• oGEM - Met with other Ontario iGEM teams
• Discussed projects, progress, shared ideas and tips

• Used iGEM Wiki Generator developed by University of Toronto
Future Work

Project
• Complete plasmid construction
• Submit new BioBrick to iGEM Registry

Hardware
• Complete prototype: narrow bandpass filters
• Develop enclosure-box that is also an incubator

Human Practices
• Open-Access Educational Blog
• oGEM Collaboration
Conclusion

• Developed a novel application for a green/red light sensitive expression system characterized in *E. Coli*
• Our system has major applications in industry and laboratory settings
• Commenced preliminary assembly of required plasmids
• Developed BioBricks currently being finalized for submission to the registry
• Created social media outreach to engage and educate the community about synthetic biology; corresponded with oGEM to spread synbio across Ontario
Acknowledgements

Supervisors and Advisors
Dr. Kimberley Dej, Dr. Rosa da Silva, Dr. Marie Elliot, Alison Cowie, Katherine Corneliaus

Sponsors

McMaster University
- Science
- Health Sciences
- Biology
- Biochemistry & Biomedical Sciences
- IDT
- ASF
- Spectrum
Thanks for listening!

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Any questions?
References

