IDEAL

PROTEIN

PURIFICATION

Researchers:
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Jeff Nivala (BioE)
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Justin Siegel
Sean Sleight
Ingrid Swanson
**Concept: Alex**
- Traditional Protein Purification
- IPP method

**Design: Chris**
- Target Vector
- Secretion Plasmid
- Display Plasmid

**Construction & Characterization: Jeff and Doug**
- New BioBricks Construction
- Target and Secretion Characterization
- Display Characterization

**Future Directions: Josef**
- Computationally Designed Biotin Binding Protein
- Conclusions
Traditional Protein Purification:

- **_clone**: afp
- **Transform**: BL21
- **Culture**: TB
- **Pellet, Lyse**: 0.5hr

**Purification Time**: 3.6 hours

- **Strip Buffer**
- **Elute 0.2hr**
- **Wash Buffer**
- **Wash 1.0hr**
- **Load Column 0.4hr**
- **Filter 0.1hr**
- **Pellet 1.0hr**

Ideal Protein Purification:
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Ideal Protein Purification:
Ideal Protein Purification:

afp: A Favorite Protein
D: Display Plasmid
S: Secretion Plasmid
T: Target Vector

Clone → Transform → Purification

Time: 10 min

TB
UW
BUFFER
1

D
S

UW5α

Culture

1700%
FASTER!!

Pellet
5 min

Pellet, Resuspend
5 min

UW BUFFER 1
**Ideal Protein Purification:**

<table>
<thead>
<tr>
<th>Advantages of the ideal protein purification system</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Can do BETWEEN CLASSES!!!</strong>&lt;br&gt;Grow, pellet, resuspend, pellet, collect...</td>
<td><strong>Cheap and Easy</strong>&lt;br&gt;No ultracentrifuge, columns, resins, etc.</td>
</tr>
<tr>
<td><strong>Expression of difficult proteins</strong>&lt;br&gt;e.g. Kinases, proteases, lipases, etc.</td>
<td><strong>Increased protein stability</strong>&lt;br&gt;Fewer proteases out side of cells</td>
</tr>
<tr>
<td><strong>Purer proteins</strong>&lt;br&gt;Fewer proteins needing separation</td>
<td><strong>Environmental Detoxification</strong>&lt;br&gt;secretion of enzymes that degrade pollutants, attack pathogens, etc</td>
</tr>
</tbody>
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Target Vector:
Target Vector:

Target Vector

BBa_K215002

PSB1A2
Target Vector:

Target Components:
- Nano-Tag: Cloning site for “afp”
- TEV: Protease Cleavage Site
- 6xH: Traditional Purification Tag
- prtB: Secretion signal sequence
- prtB: Display protein binding peptide
- Terminator_BBa_B0015: Transcription Stop

Secretion Plasmid:

Display Plasmid

Target Vector

Secretion Plasmid
Secretion Plasmid:

BBa_K215107

PSB3T5
Proteins previously shown to be secreted:

- GFP (28.5 kDa)
- Lipase (50 kDa)
- Endochitinase (42 kDa)
- β-galactosidase (3.6 kDa)
- OmpC (24.5 kDa)
- +more

Proposed Secretion Structure:

Harvard (2006) Display Plasmid:
Harvard (2006) Display Plasmid:

Harvard 2006
BBa_J36848 / BBa_J36849 / BBa_J36850 / BBa_J36851

PSB1A3
Proposed Display System Structure:
Expected Challenges:

TARGET AND SECRETION SYSTEM

- Synthesize & BioBrick a Target Vector
- Show a target fusion protein is functional
- Synthesize & BioBrick a Type I Secretion System
- Optimize an assay to quantify secreted protein
- Determine effects of extra target tags on secretion
- Optimize secretion system vector, RBS, and promoter

DISPLAY SYSTEM

- Show that legacy parts BBa_J36848-51 express protein
- Assess whether legacy parts bind biotin on cell surface
  - Fluorescence microscopy
  - Flow cytometry
- Design a custom, modular Display system
- Show protein is displayed on surface
- Explore alternative binding proteins
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Gene Synthesis by Assembly PCR:

- prtF: 1400bp
- prtD: 1800bp
- prtE: 1400bp
- TARGET: 920bp
GFP is Still Functional as Target Fusion Protein:

Fluorescence Units at Ex 485; Em 525

- GFP
- GFP + IPTG
- Target-GFP
- Target-GFP + IPTG
Secreted Protein Assay:

1. Clone
2. Transform
3. Culture, Induce
4. Spin
5. Purify Supernatant, Measure fluorescence
6. Fluorescence Conversion

Fluorescence Standard Curve:

<table>
<thead>
<tr>
<th>Protein (mg/ml)</th>
<th>Fluorescence Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.002</td>
<td>50</td>
</tr>
<tr>
<td>0.004</td>
<td>100</td>
</tr>
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</table>

Fluorescence Conversion
Amount of Target Protein in Media:

![Diagram showing the amount of target protein in media over time. The x-axis represents time in hours ranging from 0 to 50, and the y-axis represents protein in media in ng/mL ranging from 0 to 700. The diagram includes three panels with different stages of the protein release process. The top panel shows a linear relationship between time and protein concentration, with a green line indicating the trend. The middle panel shows a slightly curved relationship, and the bottom panel shows a horizontal line, indicating no change in protein concentration over time.]
Amount of Target Protein in Media:

- Fluorescent protein (ng/mL)
- Time (hrs)

Graph showing the amount of target protein in media over time.
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Confirmed Protein Expression of Legacy Parts via Western Blot:
Streptavidin Coated Beads that were a comparable size to *E. coli* were used as a positive control.
Fluorescence Microscopy Looking for Biotin Binding:

**Streptavidin Coated Beads**

**E. coli with lpp-OmpA-Streptavidin**
Flow Cytometry to Look for Biotin Binding:

Streptavidin Coated Beads that were a comparable size to *E. coli* were used as a positive control.
Flow Cytometry to Look for Biotin Binding:

Streptavidin Coated Beads

E. coli with lpp-OmpA-Streptavidin
Current Hypothesis for Non-Functional Streptavidin:

- Streptavidin is most functional as a tetramer
- Maybe as a fusion protein with OmpA
  streptavidin is not able to form tetramers.
Custom Display System (CDS):

Custom Display Plasmid

BBa_K215200 & BBa_K215201

PSB1A3
Custom Display System (CDS):

E. coli with:
lpp-OmpA-GS-LINKER-TEV-Streptavidin

Streptavidin coated beads
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Ideal Surface Display Proteins are functional as monomers
Designing a Monomeric Protein:

- Active Site
- Protein Scaffolds
- Rosetta Match
- Initial Match
- Fold-it
- Optimized Match
- Global Optimize
- Mutate Side-chain
- Local Optimize
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17 BIOBRICKS SUBMITTED
5+ WORK AS DESIGNED

4 BIOBRICKS SUBMITTED
4 LEGACY BIOBRICKS CHARACTERIZED
Thanks to...

Eric Klavins (Faculty Adviser)
Herbert Sauro (Faculty Adviser)

Graduate Students/Post Doc. who made this possible:
Justin, Ingrid, Rob, Sean and Austin

Baker Lab for Space, Equipment and General Guidance
iGEM competition for creating an undergraduate friendly environment
Electrical Engineering, Bio-Engineering and NSF for funding
Target Vector Assembly:

1. Cut with Nhel
2. PCR, Cut with X & S
3. Mix, Ligate, Screen
4. Target Vector Assembly