Beadzilllus

Fundamental BioBricks for *Bacillus subtilis* & spores as a platform for protein display
We could not have accomplished our project without our Gold Sponsors:
## Comparison to *E. coli*

<table>
<thead>
<tr>
<th>Organism</th>
<th><em>B. subtilis</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
<td>Gram + rod</td>
<td>Gram - rod</td>
</tr>
<tr>
<td><strong>Safety</strong></td>
<td>GRAS</td>
<td>GRAS</td>
</tr>
<tr>
<td><strong>Transformation</strong></td>
<td>Natural competence</td>
<td>Artificial competence</td>
</tr>
<tr>
<td><strong>Vectors</strong></td>
<td>Integrative &amp; replicative</td>
<td>Replicative</td>
</tr>
<tr>
<td><strong>Differentiation</strong></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Sporulation</strong></td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
### Lack of Bacillus Parts

#### Introduction

**Bacillus**BioBrickBox  
**Sporo**beads  
**Sporo**vector  
**Germination**STOP

---

#### iGEM Teams

<table>
<thead>
<tr>
<th>Year</th>
<th>B. subtilis Projects</th>
<th>Other</th>
<th>Total</th>
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<tbody>
<tr>
<td>2012</td>
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<td></td>
<td>184</td>
</tr>
<tr>
<td>2011</td>
<td>3</td>
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<tr>
<td>2010</td>
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<td>130</td>
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<td>2009</td>
<td>2</td>
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<tr>
<td>2008</td>
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</table>

#### Bacillus BioBricks

<table>
<thead>
<tr>
<th>Year</th>
<th>Working + Available</th>
<th>All</th>
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<tbody>
<tr>
<td>2012</td>
<td></td>
<td></td>
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<tr>
<td>2011</td>
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<td>79</td>
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<tr>
<td>2008</td>
<td>17</td>
<td>87</td>
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</table>
## Creation of 4 Working Backbones

<table>
<thead>
<tr>
<th>Description</th>
<th>Cloning status</th>
<th>Works?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Backbone (amyE, Cm)</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Backbone (thrC, Spec)</td>
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<td>✔️</td>
</tr>
<tr>
<td>Backbone (lacA, MLS)</td>
<td>in progress</td>
<td>not tested</td>
</tr>
<tr>
<td><strong>lacZ reporter</strong></td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td><strong>luxABCDE reporter</strong></td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>
Evaluation of 15 Promoters

- Anderson promoter collection
- Constitutive: $P_{\text{liaG}}$, $P_{\text{lepA}}$, $P_{\text{veg}}$
- Inducible: $P_{\text{lia1}}$

Promoter collection that spans 4 orders of magnitude!
Evaluation of 4 Novel Reporters

1. **mKate2**: Monomeric far-red fluorescent protein

   ![mKate2 Image]

3. **LacZ**: β-galactosidase

   ![LacZ Image]

2. **GFP**: green fluorescent protein

   ![GFP Image]

4. **Luc**: encodes firefly luciferase

   ![Luc Image]
## Comparison to *E. coli*

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<td><strong>Type</strong></td>
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<td><strong>Motility</strong></td>
<td>+</td>
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From Life Cycle to Sporobeads

- **Sporulation**
- **Vegetative Cycle**
- **Germination**

- **Introduction**
- **Bacillus BioBrickBox**
- **Sporobeads**
- **Sporovector**
- **Germination STOP**
- **Applications**
- **Safety**
- **Conclusion**
Resistant Structure of Spores

- **Core**
- **Cortex**
- **Inner & outer coat**
- **Crust: CgeA and CotZ**

McKenney et al., 2010
Variety of Constructs

- Integration locus
- Sporulation promoter
- Spore crust protein
- Fusion protein
- Integration locus

- *amyE*'
- *thrC*'
- *cotZ*
- *cgeA*
- *term*

CotZ CgeA
Variety of Constructs

- **Integration Locus (fwd)**
- **Sporulation Promotor**
- **Spore Crust Protein**
- **Fusion Protein**
- **Integration Locus (rev)**

**Variety of Constructs**

- $amyE'$
- $thrC'$
- $cotZ$
- $cgeA$
- $gfp$
- $term$
- $'amyE$
- $'thrC$

GFP as proof of principle
Variety of Constructs

Integration locus

Sporulation
promotor

Spore crust
protein

Fusion
protein

Integration
locus

A. cotVWXYZ cluster

B. cgeABCDE cluster
The Proof: Glowing Spores!

Introduction

Bacillus BioBrickBox

Sporobeads

Sporovector

Germination

STOP

Applications

Safety

Conclusion

The Proof: Glowing Spores!
We made it!!
Gene of interest in RFC25 (Freiburg Standard)

Sporovector: Create Your Own Bead

Gene of interest in RFC25 (Freiburg Standard)
Introduction

Bacillus BioBrickBox

Sporobeads

Sporovector

GerminationSTOP

Applications

Safety

Conclusion

Sporulation

Vegetative Cycle

Gene knockouts

Safety Applications Conclusion

GerminationSTOP

13

Gene knockouts

Vegetative Cycle
Germination Gene Knockouts

- 4 germination genes knocked out
- Knockouts combined up to quadruple mutants
- Not a single germinating spore in $3 \times 10^8$
Suicide switch

... Kill it before it spreads
Suicideswitch

... Kill it before it spreads
A New Safety Feature

IntroducNon

BacillusBioBrickBox Sporobeads Sporovector GerminationSTOP Applications Safety Conclusion

Making Bacillus a safe chassis
Safe Platform for Protein Display

Introduction  BacillusBioBrickBox  SporoBeads  Sporovector  GerminationSTOP  Applications  Safety  Conclusion

Sporulation

Vegetative Cycle

Suicideswitch

Gene knockouts
CPX - Sporobeads
Kumamolisin - Sporobeads
Students Workshop

Skinbeads

Drugbeads
Release of Sporobeads

Are Sporobeads GMOs? “ Probably not ”

Are Sporobeads organisms? “ Not really - Spores as vehicles ”
Beadzillus
Acknowledgements

• Our generous sponsors

• Our devoted advisors

• The entire Mascher Lab Group
BOSTON OR BUST!
Beadzillius

Fundamental BioBricks for *Bacillus subtilis* & spores as a platform for protein display
Creation of 4 working vectors

Ampr res

amyE

P

gene

term

Kan res

amyE

E. coli ori

AmyE

AmyE

Amp res

E. coli ori
### Creation of 4 working backbones

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</tr>
<tr>
<td><em>luxABCDE</em> reporter</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>
## Vectors

<table>
<thead>
<tr>
<th>Vectors</th>
<th>BBa#</th>
<th>B. subt. res.</th>
<th>Insertion locus</th>
<th>Description</th>
<th>Cloning status</th>
<th>Works?</th>
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</thead>
<tbody>
<tr>
<td>pSB(_{Bs})1C</td>
<td>K823023</td>
<td>Cm</td>
<td>amyE</td>
<td>empty</td>
<td></td>
<td>✔️</td>
</tr>
<tr>
<td>pSB(_{Bs})4S</td>
<td>K823022</td>
<td>Spec</td>
<td>thrC</td>
<td>empty</td>
<td></td>
<td>✔️</td>
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<tr>
<td>pSB(_{Bs})2E</td>
<td>K823027</td>
<td>MLS</td>
<td>lacA</td>
<td>empty</td>
<td>in progress</td>
<td>not tested</td>
</tr>
<tr>
<td>pSB(_{Bs})1C-lacZ</td>
<td>K823021</td>
<td>Cm</td>
<td>amyE</td>
<td>lacZ reporter</td>
<td></td>
<td>✔️</td>
</tr>
<tr>
<td>pSB(_{Bs})3C-luxABCDE</td>
<td>K823025</td>
<td>Cm</td>
<td>sacA</td>
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<td></td>
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</tr>
<tr>
<td>pSB(<em>{Bs})4S-P(</em>{xyl})</td>
<td>K823024</td>
<td>Spec</td>
<td>thrC</td>
<td>xylose-promoter</td>
<td>refuses trafo</td>
<td></td>
</tr>
<tr>
<td>pSB(<em>{Bs})0K-P(</em>{spac})</td>
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<td>Kan</td>
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<td>IPTG-promoter</td>
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<tr>
<td>Sporo vector</td>
<td>K823054</td>
<td>Spec</td>
<td>thrC</td>
<td>for Sporo beads</td>
<td></td>
<td>not tested</td>
</tr>
</tbody>
</table>

![Diagram of pSB\(_{Bs}\)3C-luxABCDE vector](image.png)
Promoters – Anderson promoters

Bacillus BioBrickBox

Introduction  Sporo beads  Sporo vector  Germination STOP  Applications  Safety  Conclusion

Promoters

- Anderson promoters

Bacillus BioBrickBox
**Promoters - Constitutive**

### Introduction

- **BacillusBioBrickBox**
- **Sporobeads**
- **Sporovector**
- **Germination**
- **Applications**
- **Safety**
- **Conclusion**

---

**:P_{liaG}**

- **OD_{600}**
  - **Time [h]**
  - **τ = 51.1 min**
  - **τ = 53.9 min**

**:P_{lepA}**

- **OD_{600}**
  - **Time [h]**
  - **τ = 55.6 min**
  - **τ = 51 min**

---

**Safety Applications**
Promoters - Constitutive

- $P_{liaG}$
- $P_{veg}$

![Graph showing Miller Units and OD600 over time for $P_{veg}$ and $P_{liaG}$](image-url)
Promoters - Inducible

$P_{lia}$
Promoters - Inducible

-\[ P_{lia} \]

<table>
<thead>
<tr>
<th>Miller Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>time (Min after Induction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>120</td>
</tr>
</tbody>
</table>

-\[ P_{lia} \] ind.
-\[ P_{lia} \] not ind.
Promoters - Overview

- Bacillus BioBrickBox
- Sporo beads
- Sporo vector
- Germination STOP
- Applications
- Safety
- Conclusion

Promoters Overview

Bacillus

B

B

B

Box

RLU/OD (OD=0.1)

10^6

10^5

10^4

10^3

10^2

10^1

#103 #113 #115 #117 #118 #106 #100 #107 #102 #114 #101 P_{\text{lia}A} P_{\text{lia}G} P_{\text{lepA}} P_{\text{veg}} P_{\text{lia}A} (+bac)

Anderson promoters

P_{\text{lia}A} (-bac)
### GFP SporoBead Data

<table>
<thead>
<tr>
<th>Strain</th>
<th>genetic map / average intensity</th>
<th>phase contrast / fluorescence</th>
<th>3D-surface plot</th>
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</thead>
<tbody>
<tr>
<td>W168</td>
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<td><img src="image3" alt="3D Surface" /></td>
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<td>B 73</td>
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<tr>
<td>B 55</td>
<td><img src="image1" alt="Genetic Map" /></td>
<td><img src="image2" alt="Phase Contrast" /></td>
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<tr>
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<td><img src="image2" alt="Phase Contrast" /></td>
<td><img src="image3" alt="3D Surface" /></td>
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<tr>
<td>B 54</td>
<td><img src="image1" alt="Genetic Map" /></td>
<td><img src="image2" alt="Phase Contrast" /></td>
<td><img src="image3" alt="3D Surface" /></td>
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<tr>
<td>B 71</td>
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<td><img src="image2" alt="Phase Contrast" /></td>
<td><img src="image3" alt="3D Surface" /></td>
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<td><img src="image1" alt="Genetic Map" /></td>
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<td>B 70</td>
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<td><img src="image2" alt="Phase Contrast" /></td>
<td><img src="image3" alt="3D Surface" /></td>
</tr>
</tbody>
</table>
Spore Purification

B53-untreated

B53-French Press

B53-Sonification

B53-lysozyme
Spore Purification

W168-lysozyme

B53-lysozyme
Cells grow into colonies, spores unable to germinate

Cultures in DSM

Heat

Cells die, spores survive

No heat

Cells survive, spores survive

Spores germinate into colonies

WT

Spores unable to germinate

Mutant

Cells grow into colonies, spores germinate into colonies

Cells grow into colonies, spores unable to germinate
Gene knock-outs

Mutant Germination Rates

Percentage of spores germinated in logarithmic scale

W168
spooA::tet
B29
B30
B32
B40
B41
B42
B43
B46
B47
Double mutants
Triple mutants
Quadruple mutants
Human Practice Projects

SynBio Conference
German iGEM meeting

Germany-wide SynBio Day

High school Practical Course
Beadzillus

Fundamental BioBricks for *Bacillus subtilis* & spores as a platform for protein display