GENOMIKON

Unlock the secrets of the Codex

Everything you need to build, test and operate genetic circuits

Alberta iGEM 2010
Meet the Team: Alberta iGEM 2010

22 undergraduates (2\textsuperscript{nd}-4\textsuperscript{th} year) from:
the faculties of Science, Engineering, Medicine, Business and Fine Arts
Acceleration: SynBio must provide the means to **rapidly** design, test and optimize living systems.
Acceleration: Technical and Cultural

**Technology:** Getting results fast

- Process speed
- Minimizing process error
- Minimizing user error

**Culture:** Access to technology increases understanding of it.

- Affordability
- Reliability
- Simplicity
But......

What if there was a piece of technology that could answer both of these needs?

That could be revolutionary!
Introducing GENOMIKON

A self-contained SynBio kit targeted for high school and first year university students

- Enough parts for 100s of meaningful experiments
- Experiments can be completed in an afternoon
- Inexpensive!
- No lab equipment required
- An online social network resource:
  - Lab manual
  - Notebook
  - Info management
  - Sharing ideas
Accelerating Assembly

BioBricks:

Advantages:
- Modular
- Standardized
- Lots of part choices

Limitations
- Slow

3 days
Accelerating Assembly

1st Byte

Ligase + Byte #1

Repeat....

35 min.

7 minutes

BioBricks:

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Accelerating Assembly

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BioBricks:

Advantages:
- Modular
- Standardized
- Lots of part choices

Limitations
- Slow
Controlling Byte Order, Orientation and Copy Number

BioBrick overhangs:
- Symmetrical and complementary
- Mixed products after one cycle
Controlling Byte Order, Orientation and Copy Number

The solution: Special overhangs

**BioByte overhangs:**
- Nonidentical and non-symmetric
- Mixed products after one cycle

Two Byte Formats

- AB
- BA

Self-Ligation

Cycle 1
- Fixed Orientation
- Single copy addition

Cycle 2

A B A' B'
Functional Nature of Bytes

- Promoters
- RBS
- Terminators
- mRNA Linkers
- End inverters

Linkers Define the 1\textsuperscript{st} and Last Codons

Linker
ORF
Linker
ORF
Linker

BA
AB
BA
AB
BA

x
Met
N and C terminal
Protein Fusions and Tags

y
Stop
MOLECULAR DETAIL
PCR with universal primers

BsaI: an offset cutter
Leaving a 4 base overhang

BioByte Production

pSB1C3-AB

pSB1C3-BA
Anchor and Cap

- **Iron Bead**
- **dT**
- **25**
- **dA**
- **18**
- **BsaI**

- **Anchor and Cap**
  - **Ligate to 1st Byte**
  - **Remove access anchor**

- **5 min @ 75°C**
- **Room Temp**

- **Cool & Transform**
- **No ligation!**
TESTING
Experiment 1: Fidelity of Ligation

Fidelity & Efficiency confirmed
Experiment 2: 3 Steps of Assembly

A. Anchor binding and release

B. 2nd Byte addition and release

Supernatant Wash 75°C
Sup 75°C

Chain initiation, extension & release
Experiment 3: Coupling Efficiency

Building a 12kB Octamer

- 50% coupling Efficiency
- final yield: <<<
- 93% coupling Efficiency
- 63% final yield

BioBytes 2.0
- 93% coupling Efficiency
- 63% final yield

BioBytes 1.0
- 50% coupling Efficiency
- final yield: <<<

X10?
Experiment 4: Cap – Anchor Selection

5 part Assembly

Cap+

Anchor     Ori     RFP     KanR     Cap

Cap-

Anchor-to-Cap circularization provides strong selection for full-length product
THE KIT
- Introduce students to the kit
- Provide a foundation in synthetic biology
- Empower students to create and complete their own experiments
Lab Manual: Experiments

Designed to:

- Use only materials available in the kit
- Teach concepts that correspond with a high school biology curriculum
- Go beyond “learning” and start “doing”

Examples Experiments Include:

- Fluorescent bacteria,
- Color readouts
- Genetic circuits
Bacteria that remember

admin

Bacteria -- simple blobs of metabolizing chemicals, reacting solely to their environment? Not so! Bacteria can actually be programmed with a biological circuit to remember, and pass this memory down, mother-to-daughter, over generations. In this experiment, we'll build a flip-flop memory into a cell.

**Constructs:**

**Toggle Switch**

![Diagram of a biological circuit](image)

1 **Mix**

Mix the iron micro beads for 10 minutes.

2 **Transfer Beads**

Transfer a 25 ul (one drop) aliquot of iron micro beads to a 1.5 mL tube.
Look at your colonies of bacteria under a black light.
Use the provided UV flashlight to examine the colonies you grew.

This experiment was cool, my colonies are glowing!
Proteins

Because they are so small, most people see cells as stationary life forms which go through mitosis every once in a while, but cells do so much more! They eat, drink, swim towards food and away from trouble - they can even “talk” to other cells. How do cells do all this if they don't have stomachs or muscles like other animals? They use proteins! Proteins are large strings of molecules known as amino acids. There are 20 types of amino acids and while that may seem like a small number, they can have millions of different combinations. Hundreds of these amino acids join together and fold into different complex shapes that allow them to do their unique job. The different characteristics of the amino acids in a protein determine how that protein will interact with its environment. Some proteins are highly charged and work with oppositely charged molecules. Others are very stable and act as holes in the cell’s membrane allowing nutrients to come into the bacteria. Some release chemicals and others repair DNA. Because there are so many different ways we can combine amino acids, there are tons of things a new protein could do.
Experiment Designer tool
Automatic Sequence Generation

Sequence:
Length: 4595 bp

```
0: TGCGCGACTGAGCGCAGCGATCGAAGATTCTGCTCTACTACGCAACGCGACTCTCTCCCGCGCTTGGCGGATTGTCAAAAGCTGACGTGGCAAGCGTT
0: ACCGCGACTGAGCGCAGCGATCGAAGATTCTGCTCTACTACGCAACGCGACTCTCTCCCGCGCTTGGCGGATTGTCAAAAGCTGACGTGGCAAGCGTT

P_lac

SC101 Ori  B  Lac Promoter Cassette

n-vioA

Lac Promoter Cassette  A

RBS

Lac Promoter Cassette

```

**Generate a Designool**

**Bacteria that can be created**

** Constructs:**

1. **Mix**
   Mix the post-translational proteins for 10 minutes.

2. **Transfer Beads**
   Transfer a 50 ul (one drop) aliquot of post-translational proteins to a 1.5 mL tube.

3. **Pull Beads**
   Pull the beads to the side using the magnetic tube rack supplied. Remove and discard the supernatant.

4. **Wash**
   Add 50 ul (0.5 drops) of supplied Wash Buffer  to the beads. Flick gently to resuspend. Pull the beads to the side using the magnetic tube rack. Remove and discard the supernatant.

5. **Re-Wash**
   Add 50 ul (0.5 drops) of supplied Wash Buffer  to the beads. Flick gently to resuspend. Pull the beads to the side using the magnetic tube rack. Remove and discard the supernatant.

6. **Add Anchor**
   Add 200 ng of a pre-made anchor oligo construct to the beads and top off the reaction with water. Flick gently to resuspend.

7. **Anneal**
   Allow annealing for 30 minutes, mixing by flicking every 5 minutes. Ensure that there are no droplets on the sides of the tube.
Leading edge of bio lab kits
Natural fit in the high school biology curricula
Introduce 1000s of students to synthetic biology
"I learned the theory already, but I got to apply it today."

– Aymen Saidane, 17
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4 SMs \times 3 \text{ rep ORIs} \times 9 \text{ promoters} \times 8 \text{ reporters} > 864 \text{ different plasmids}
Enough precision droppers for an entire class $3
UV flashlight $15
Iron Microbeads $4
Magnetic racks $7

Making Synthetic biology accessible priceless
Thanks To Our Generous Contributors
### Materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
<th>Cost</th>
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</thead>
<tbody>
<tr>
<td>E. coli CC</td>
<td>18</td>
<td>20.00</td>
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<tr>
<td>Magnetic Racks</td>
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<td>7.50</td>
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<tr>
<td>Precision Dropper</td>
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<td>2.99</td>
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<tr>
<td>LB Plates</td>
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<td>1.00</td>
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<tr>
<td>Tubes</td>
<td>18</td>
<td>1.00</td>
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<tr>
<td>Buffer Elution</td>
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<td>0.50</td>
</tr>
<tr>
<td>Buffer Wash</td>
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<td>0.50</td>
</tr>
<tr>
<td>Magnetic Microbeads (ml)</td>
<td>6</td>
<td>4.20</td>
</tr>
<tr>
<td>DNA parts</td>
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<td>3.00</td>
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</tbody>
</table>

### Labour

- Stipend Employee (h) @ 15.00: 0.75 @ 15.00 = 15.00

### Variable Overhead

- Packaging: 3.14
- Mailing: 13.16

### Total Incremental Cost

- **Total cost of parts 71.99**
Modelling efficiency