iGEM TEAM HEIDELBERG 09 SPYBRICKS FOR YOUR EYES ONLY
VIROTHERAPY
Smart viruses finding & killing malignant tissue

SYNTHETIC TISSUE
Hearts from the flask

SMART CELL LINE
Cells discovering new drugs
Parts Registry

Only 13 teams out of 304 working on mammalian systems
IMAGINE...
Towards Synthetic Mammalian Biology

TEAM Heidelberg ’09: OUR MISSION
A STANDARD for MAMMALIAN BIOBRICKS
OUR AIM

- Complexity
- Compartimentalization
- Transcriptional Control
- Transcription Factors
- Signaling Pathways
- Promoters
Complexity

Transcription Factors

Promoting Standardized Synthetic Mammalian Biology

CREATE SYNTHETIC PROMOTERS

Transcriptional Control

Promoters
• Standardized Measurement
• Cloning Standard
Standardized Measurements in Mammalian Cells

- RNA Processing
- RNA Export
- Protein Folding
JeT: an existing synthetic promoter

\[
\begin{align*}
&\text{EcoRI} \quad \text{Sp1} \quad \text{Sp1} \quad \text{CCAAT} \\
1 & \text{GAATT} \text{GGGCC} \text{AGTTA} \text{GGGCG} \text{AG} \text{CCAAT} \text{CAGCGTGCGCCGTTCGAAA} \\
51 & \text{GGGCC} \text{AGAAT} \text{GGGCG} \text{GTAACGCGATGATTAT} \\
& \text{TTGCGTTTATGGCTGGCGCAGAAT} \\
& \text{ATAAGGACGCGCCCGGTTGTGGCACAGCTAGTT} \\
& \text{GGGTCCGGTCTTGTTTGTGGATCCTGTGACTGTCACCTTGACA} \\
\end{align*}
\]

modified from Tornoe et al. 2002
Measurement
Introducing New Measurement Units

Protein level

mRNA level

Relative Expression Unit (REU)

\[
\text{Relative Expression Unit (REU)} = \frac{P_x}{P_{JeT}}
\]

P = total protein level

Relative Mammalian Promoter Unit (RMPU)

\[
\text{Relative activity of promoter } x \text{ (RMPU)} = \frac{PoPS_x}{PoPS_{JeT}} = \frac{M_x}{M_{JeT}}
\]

M = total mRNA level; PoPS = Polymerase Per Second
Measurement
Comparing Different Techniques

Protein level
mRNA level
Microscopy
Flow Cytometry
qRT-PCR

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CMV Characterization
Applying New Measurement Units

- Protein level
- mRNA level

## Microscopy

<table>
<thead>
<tr>
<th>Method</th>
<th>CMV</th>
<th>JeT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>7.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>5.5</td>
<td>2.9</td>
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## qRT-PCR

<table>
<thead>
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<th>Method</th>
<th>CMV</th>
<th>JeT</th>
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<tbody>
<tr>
<td>qRT-PCR</td>
<td>2.9</td>
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</tr>
</tbody>
</table>
CMV Characterization
Applying New Measurement Units

Protein level
- Microscopy
- Flow Cytometry

mRNA level
- qRT-PCR

Graph showing REU levels for HeLa, MCF-7, and U2-OS cell lines:
- HeLa: 5.5 REU
- MCF-7: 6.6 REU
- U2-OS: 10.0 REU

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Towards Mammalian BioBricks
Why should we use BioBrick Beta?

**Cloning Standards**

**Bacterial parts:** BioBrick alpha
- ✓ High parts number
- ✗ No protein fusion

**Mammalian parts:** BioBrick beta
- ✓ Virtually no parts
- ✓ Protein fusion

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Towards Mammalian BioBricks

Why should we use BioBrick Beta?
CLONING STANDARD

SYNTHETIC MAMMALIAN BIOLOGY

STANDARDIZED MEASUREMENT & DEVICES
Synthetic Promoters
Random Approach

FOUNDATIONS
RANDOM
RATIONAL
Synthetic Promoters
Random Approach

• Generate randomized promoters
• Comprehensive model of promoter structure
Synthetic Promoters
Random Approach

- Generate randomized promoters
Promoter – Model
Describing Promoter Composition

Proximal promoter = regulated
Core promoter

Spacer sequence TFBS TFBS

TFBS = Transcription Factor Binding Site
Random Assembly PCR
Six Steps to a Random Promoter

1. Oligonucleotides ("Oligos") Design

2. Annealing
Random Assembly PCR
Six Steps to a Random Promoter

3. Seven Cycle PCR / 4. 25 Cycle PCR
Random Assembly PCR
Gradually from Low to High Strength

5. Cloning

6. Screening in mammalian cells
Constitutive Promoter Library
Gradually from Low to High Strength

6. Screening in mammalian cells
RA-PCR: Results
Library of Inducible NFkB Promoters

NFkB is induced by TNFα

Uninduced
Induced

Arbitrary unit (a.u.)

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RA-PCR Results: Time-Lapse Inducing NFkB Responsible Promoter

- Accurate REU measurement by FC
- Confirmed that promoter is only induced by NFkB, not by other TFs

GREEN: GFP in cytoplasm
RED: Constitutive mCherry in the plasma membrane

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RA-PCR Results: Time-Lapse
Inducing NFkB Responsible Promoter

RA-PCR: Powerful method - creates entire libraries of promoters!

- Accurate REU measurement by FC
- Confirmed that promoter is only induced by NFkB, not by other TFs

GREEN: GFP in cytoplasm
RED: constitutive mCherry in the plasma membrane

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Synthetic Promoters
Rational Design - HEARTBEAT

HEARTBEAT

- Heidelberg Artificial Transcription factor Binding site Engineering and Assembly Tool -
Definition of model parameters

- Positional Preference of the Transcription Factor Binding Site (TFBS)
- Co-occurrence of defined Transcription Factors
- Transcription Factor Binding Affinity
Parameter Acquisition

HEARTBEAT Database

>4000 human promoter sequences

- Frequency distribution

- Co-occurring TFs

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Parameter Acquisition
HEARTBEAT Database

>4000 human promoter sequences

Spatial preference
Transcription factor co-occurrence

Binding motif: Consensus sequence

Quality of spacer sequence

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We designed 12 sequences containing SREBP binding sites including 3 negative controls. 6 sequences exhibit supportive Sp1 binding sites, and 2 were responsive to sterol depletion.

SREBP (Sterol Regulatory Element Binding Protein) is active upon sterol depletion.
Experimental Validation
HEARTBEAT Database

We designed 12 sequences containing SREBP binding sites including 3 negative controls.

6 sequences exhibit supportive Sp1 binding sites.
2 were responsive to sterol depletion.

SREBP
(Sterol Regulatory Element Binding Protein)
is active upon sterol depletion.

Sequences that fit our model best work!
Definition of input parameters

General Features
Length promoter: 500 bp
Core promoter properties
Transcription factor 1
TF: SREBP
Co-TF: Sp1
Graphical User Interface
Output: the desired Sequence

Output

General Features
Length promoter: 500 bp
Core promoter properties
Transcription factor 1
TF: SREBP
Co-TF: Sp1

Visit our GUI on
http://www.HEARTBEAT-GUI.de/
Simulating Promoter Activity

Quantitative predictions from:
- experimental data
- prior knowledge
- HEARTBEAT sequences

Rule-based descriptive language

HEARTBEAT Fuzzy Network

INPUT

OUTPUT

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HEARTBEAT FN can be used for:

- Error checking
- Characterization of newly synthesized promoter parts
- Predicting combined activity
Synthetic Promoters in a Nutshell
A self-iterative Process

Sequences created by Random Assembly PCR

Optimization

Fuzzy Logic Network Modelling

Also allows

>4000 human promoter sequences

HEARTBEAT DB

Can be accessed via

HEARTBEAT GUI

HEARTBEAT GUI

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Towards iGEM 20XX

The Future Synthetic Mammalian Biology

RFC 41
standardized measurements

RFC 45
tested cloning standard for mammals

RFC 42/43
random assembly and rational design of promoters

Thousands of Mammalian Parts on the Registry

Smart Cell Line

Synthetic Tissue

Virotherapy

11/01/2009

iGEM TEAM HEIDELBERG 2009
Heidelberg says: “THANK YOU, iGEM!!!”

We are:
Michael Bartoschek, Simon Haas, Tim Heinemann, Corinna Hiller, Nao Iwamoto, Stephen Krämer, Hannah Meyer, Douaa Mugahid, Anne Rademacher, Hannah Uckelmann, Lars Velten, Bingqing Zhao, Chenchen Zhu

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Visit our wiki on http://2009.igem.org/Team:Heidelberg