THE VECTOR-JECTOR
Controlled trans-kingdom genetic transfer from *E. coli* into *S. cerevisiae*
Gene therapy transfers functional DNA to repair a defect or confer some novel ability.

Here, let me send you the latest codec.

Aw, man, my phone won’t play this video!
Overview

• Background
• Genetic Circuitry
• Device Construction
• SeToB – Web-based Biological Circuit Designer
Inspiration: Bacteria can transfer DNA to yeast by conjugation*

Goal: Control DNA transfer from bacteria to yeast

Specifications:

1. Yeast requires a new genetic ability

2. Bacteria contains a plasmid with gene(s) that confer the required ability

3. Bacteria conditionally transfers ability-conferring plasmid to yeast
Design: Bacteria transfers DNA to yeast under certain conditions

HELP!

Vector-Jector to the rescue!

E. coli

S. cerevisiae
Specifics: *E. coli* transfers DNA to distressed *S. cerevisiae* under certain conditions

Specifications:

1. Yeast requires the ability to digest lactose

2. Bacteria contains a plasmid with the *lacZ* gene, which imparts the ability to digest lactose in yeast

3. Bacteria conditionally transfers ability-conferring plasmid to yeast when lactose is present, glucose is absent, and distressed yeast is in close proximity.
Vector-Jector Design: High-Level Architecture

- Environment Sensing Module
  - Input [X] Lactose
  - Input [Y] Glucose
- Conjugation Machinery Control Module
  - On/Off PoPS
- Signaling Module
  - Signal AHL
- Conferred Ability Module
  - Conferred Ability
  - β-galactosidase

E. coli S. cerevisiae
Vector-Jector Design: High-Level Architecture

- **Input [X]**: Lactose
- **Input [Y]**: Glucose

**E. coli**
- 100% On/Off
- 35% Shuttle Plasmid Transfer

**S. cerevisiae**
- 10% Signaling Module
- 50% Conferrred Ability

- Signal
- AHL
- PoPS

**Conferred Ability**
- β-galactosidase

**Vector-Jector Design**
- High-Level Architecture
- Environment Sensing Module
- Signaling Module
- Conjugation Machinery Control Module
Challenges: Controlled DNA transfer from bacteria to yeast

- Construct Signaling Module
- Construct Environment Sensing Module (lactose/glucose)
- Construct Environment Sensing Module (aTc/arabinose)
- Construct Conjugation Machinery Control Module
- Standardize bacteria-yeast transfer plasmid
- Experimentally reproduce bacteria-yeast genetic transfer
- Use Conferred Ability Module to digest lactose
- Model our system mathematically
- Contribute BioBricks to the Registry
- Characterize BioBrick parts
  - BBa_R0010 pLac promoter
  - aTc/arabinose hybrid promoter (from Elowitz lab)
- SeToB: Make circuit design from BioBricks easier
- Have fun!
Signaling Module produces AHL distress signal in absence of glucose.
Signaling Module produces AHL distress signal in the absence of glucose
Environment Sensing Module produces PoPS output when environmental conditions are met.
Environment Sensing Module produces PoPS output when environmental conditions are met.

- Glucose
- Lactose
- AHL
- AND
- LuxR
- On/Off
- pLux PoPs

Input 1

Input 2

AHL Signal
Environment Sensing Module produces PoPS output when environmental conditions are met.

- Input 1: Glucose
- Input 2: Lactose

**Glucose-responsive site**

**-35 -10 LacI binding site**

**luxR**

**LuxR/AHL -35 -10**

**PoPS**

**LuxR generator regulated by Lac Promoter**

**pLac (R0010)**

**LuxR generator**

**pLux (R0062)**
Characterization of native lac promoter shows AND-gate functionality

Measurement protocols were verified with Jason Kelly’s Measurement Kit
Alternative Environmental Sensing Module component responds to different inputs

"Programming gene expression with combinatorial promoters" by Robert Sidney Cox, III, Michael G Surette, and Michael B Elowitz
Conjugation Machinery Control Module induces conjugation in response to PoPS input.
Conjugation Machinery Control Module induces conjugation in response to PoPS input.
Conjugation Machinery Control Module induces conjugation in response to PoPS input.
Conjugation Machinery Control Module induces conjugation in response to PoPS input.
Two approaches towards construction of Conjugation Machinery Control Module

Native Promoter Swapping

Knockout and Complementation

*KorA and TrbA have been BioBricked
Yeast Shuttle Vector requires five features for trans-kingdom transfer:

- **Conferred Ability Gene** (*lacZ*)
- **pAC88**
- **Yeast origin of replication**
- **Yeast selectable marker**
- **Origin of transfer**
- **Bacterial origin of replication**
- **Bacterial selectable marker**

The diagram illustrates the integration of these features into the shuttle vector.
Yeast Shuttle Vector requires five features for trans-kingdom transfer

Bacterial origin of replication

Bacterial selectable marker

Origin of transfer

BioBrick standard insertion site

Standardized Yeast Shuttle Vector

Yeast origin of replication

Yeast selectable marker
Conferred Ability Module expresses *lacZ* gene on shuttle vector to digest lactose

- **E. coli**
- **S. cerevisiae**

**Input [X]**: 
- Lactose
- On/Off
- Environmental Sensing Module
- PoPS
- Conjugation Machinery Control Module

**Input [Y]**: 
- ~Glucose
- Conferred Ability
- Conferred Ability Module
- β-galactosidase
- Ability to Digest Lactose

**Signaling Module**:
- AHL
- Signal
- Shuttle Plasmid Transfer
- PoPS

**Shuttle Plasmid Transfer**:
- Conferred Ability to Digest Lactose
- Conferred Ability Module expresses *lacZ* gene

**Environment Sensing Module**:
- Input [X]
- Lactose
- ~Glucose
Conferred Ability Module expresses *lacZ* gene on shuttle vector to digest lactose
Demonstration of bacteria-yeast conjugation: yeast gains the ability to synthesize leucine.

No conjugative plasmid present in *E. coli*  

Conjugative plasmid present in *E. coli*
Vector-Jector Design: High-Level Architecture

E. coli  S. cerevisiae

- Glucose
  Input [X]  Lactose

Environment Sensing Module

On/Off  PoPS

Conjugation Machinery Control Module

Signal

AHL

Shuttle Plasmid Transfer

Conferred Ability Module

Input [Y]

- Glucose

Conferred Ability

β-galactosidase
• Goal: Drag and drop construction of genetic networks
• Converts Registry data to PoBoL format
• Talk to Param and Tyler at poster
Accomplishments: Controlled DNA transfer from bacteria to yeast

- Construct Signaling Module
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- SeToB: Make circuit design from BioBricks easier
- Have fun!
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Environment Sensing Module

On/Off

PoPS

Conjugation Machinery Control Module

Input [X]

Lactose

~Glucose

Input [Y]

~Glucose

Signal

AHL

Shuttle Plasmid Transfer

Signaling Module

Conferred Ability Module

Conferred Ability

β-galactosidase

E. coli

S. cerevisiae