A fully automated system for polypeptide release in the small intestine

Team UW–Madison 2010
iDIET in Brief

- Growth
- Dietary Ingestion
- Acid Survival
- Lysis & Product Release
- Product Absorption
Genetic Disorders & More: Celiac Disease

- 1 in 133 are diagnosed in the US
- US healthcare cost: $14.5 - $34.8 billion annually
- No cure
- Extreme dietary measures
Genetic Disorders & More: High Cholesterol

- Cholesterol made from bile acids
- Bile Salt Hydrolase enzyme deconjugates
- Deconjugates bile salts cannot be reused
Genetic Disorders & More: High Cholesterol

Cholesterol → Bile Salts → Deconjugated Bile Salts → Bile Salts
Genetic Disorders & More: High Cholesterol

- Serum Cholesterol
- Cholesterol
- Bile Salts
- Deconjugated Bile Salts
- Bile Salts
- Excreted
Genetic Disorders & More: Lactose Intolerance

• Unable to digest lactose found in dairy products

• Model system for experiment
  – Deliver beta-galactosidase by timed-lysis to small intestines
  – Interchangeable system
Growing Micro-Organisms
Growth: Encapsulation & Enzyme Production

- RcsA
- RcsB
- B-Gal

**Colonic Acid - Encapsulation**

**Enzyme**

**constitutive**
Encapsulation Testing Constructs

**Constitutive**

- RcsA
- RcsB
- B-Gal (REPLACABLE ENZYME)

**Testing Constructs**

- **IPTG Inducible**
  - RcsA
  - RcsB
  - RcsA
  - RcsB
Encapsulation: Proof of Colonic Acid Production

Colonic Acid
Encapsulation:
Proof of Colonic Acid Production

Quantification of L-Fucose

(\text{ug/ml})/\text{OD600 of L-Fucose}

- Control
- RcsB
- RcsA
Encapsulation: Cell Survivability in Low pH

Cells Survivability at pH4

CFU per ml

- **RcsA**
- **RcsB**
- **RcsA+RcsB**
- **Control**
Enzyme Release in Small Intestine

1) Inducible Repressible
   • pH promoter
   • Activator and Repressor
   • A promoter sensitive to activator and repressor

2) Bile Salt Inducible Expression
   • *Salmonella enterica* strain LT2
   • PCR Amplify and clone *ramA* and *acrRA* genes

3) Encryption System
   • See our poster
Inducible-Repressible Lysis

Before Stomach

Stomach

Duodenum of Small Intestine

Low pH

pH

LVA

Cl

LuxR

Lysis Cassette

Cell Lysis

I/R
Inducible-Repressible Lysis: Testing Constructs

Testing Construct
Protein expression behind the gadA promoter is dependant on growth phase

- Tight control of expression below OD 3.0 (indistinguishable from baseline fluorescence)
- Limited expression from OD 3.0 to OD 5.5
- Induction as culture approaches a true stationary state

**Graphs:**
- **gadAp RFP Expression vs Time and OD (pH 7):**
  - 5x increase in expression from OD 3.0 to OD 5.5
- **gadAp RFP Expression vs Time and OD (pH 5.5):**
  - Comparison of expression profiles at different pH levels.

**Legend:**
- **gadAp + mRFP1, pSB1A2 MG1655**
- **WT MG1655**
- **placl + mRFP1, pSB1A2 MG1655**
2. Bile Inducible Lysis: System

Before Duodenum

Duodenum of Small Intestine

Constitutive

AcrRA

Cell Lysis

RamA

Lysis Cassette
2. Bile Induced Lysis: Testing Constructs

Testing Constructs

*constitutive*

*RamA* → *Lysis Cassette*

*AcrRA* → *RFP*
Enzyme Action
Large Intestine
Large Intestine
Registry Maintenance: BBF RFC 67

GENBANK

EXPERIENCE

SEQUENCING

TESTING

ATCG
Achievements

**Lysis Systems in Small Intestine**
1. Inducible-Repressible System
2. Induction in the presence of Bile
3. Encryption (see wiki/poster)

**Registry Additions**
- K318500 *
- K318501 *
- K318502 *
- K318506
- K318507
- K318511
- K318512 *
- K318513 *

* Characterized

**Characterization**
1. gadA promoter
   - OD
   - pH
2. Expression of RcsA, RcsB, RcsA&RcsB
   - Colonic acid production
   - Cell survivability

**Applications**
1. More efficient delivery of functional enzyme to the small intestine
2. Decrease in cost of production and purification for pharmaceuticals
3. Accessibility of treatments for genetic disorders
REFERENCES

IMAGES
http://diet.lovetoknow.com/wiki/Lactose_Intolerance_Symptoms
http://topnews.in/health/files/heart-attacks_0.jpg

BACKGROUND

BILE INDUCTION

INDUCIBLE/REPRESSIBLE
Questions?