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Diauxie Elimination: Increasing bioenergy efficiency

Bio-Dosimeter: Bacterial Radiation Sensing
Diauxie Background

- Traditional energy sources are not sustainable
- Biomass is a renewable resource
- Process still not efficient enough to be widely used
Diauxie Background

- Diauxie – cells growing in sugar mixtures will metabolize them one at a time.
• Eliminating Diauxie could increase the efficiency of bioreactors..
Xylose Metabolism

D-xylose

cAMP

xyLR

xyLF

xyLG

xyLH

xyRA

xyRB

xyRCR
Xylose Metabolism

**D-xylose**

$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{Phosphate}$

**XylB** | **XylA** | **XylR** | **Crp** | **XylR** | **XylF** | **XylG** | **XylH** | **XylR**
Xylose Metabolism

Xylose metabolism involves several key enzymes and regulatory proteins. The pathway begins with the uptake of D-xylose, followed by the expression of enzymes xylA and xylB. These enzymes catalyze the conversion of D-xylose to D-xylulose and then to D-xylulose-5-phosphate. The regulatory proteins crp and xylR play crucial roles in controlling the expression of these enzymes.

- **xylB**: Encodes an enzyme involved in the initial steps of xylose metabolism.
- **xylA**: Encodes an enzyme that converts D-xylulose to D-xylulose-5-phosphate.
- **xylR**: A transcriptional activator that regulates the expression of xylA.
- **crp**: A cyclic AMP receptor protein that acts as a regulator in cell metabolism.

The pathway illustrates the metabolic conversion of xylose to a form that can be further metabolized by the cell.
Strategy 1 – Regulatory Region

xylB  xylA  xylR  crp  xylR  xylF  xylG  xylH

T T G A G C ... A A T A T T
T T G A C A ... T A T A AT

T T G T T T ... T A A A A A A
T T G A C A ... T A T A AT
Strategy 1 – Testing

M9 minimal media w/ Xylose & Glucose
Strategy 1 – Final Construct
Strategy 2- CRP*

PCR Complete

Part Submitted
Strategy 2 - Testing
Strategy 2 - Final Construct
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Bio-Dosimeter:
Biological Radiation Sensing
Introduction: Dosimetry

- Exposure to radiation or radioactive material must be quantified to avoid harmful/lethal doses of radiation.
- For individuals with high exposure, electrometer dosimeter pen is an expensive and accurate way to measure dose.
- Relatively Inexpensive photographic or thermoluminescent dosimeter badges also exist.
Expanding Need for Dosimeters

• Expanding need for radiation detection
  – Homeland security

• Possible exposure to radioactive material
  – Yucca Mountain

• Organisms could be used as a first warning dosimeter

• A biological dosimeter would be cheap, easy to read, easy to maintain, but probably inaccurate
Theory of a Bio-Dosimeter

- Radiation’s harmful effects stem from the genetic damage caused by ionizing radiation
- Biological organisms already have very advanced genetic repair mechanisms
- Most direct implementation of a biosensor would monitor the genetic damage accumulated by a bacterial cell and emit a signal after a critical threshold
• Genetic Damage to bacterial host triggers λ lysogen’s entrance into lytic phase
  - Bacterial RecA recognizes mutations, cleaves λ repressor
• By utilizing key regions of the λ genome, we assemble a dosimeter ‘switch’ that would throw after a certain dosage of radiation
The lambda phage maintains lysogeny through a single bidirectional operator ($O_R$)

- Operator is controlled by two proteins that bind to three sites ($O_{R1}$, $O_{R2}$, and $O_{R3}$)

$O_R$ contains two promoters $P_{RM}$ and $P_R$ that transcribe a repressor (CI) and anti-repressor (Cro), respectively
• Trigger on cellular level is all or nothing
• Test different Cro RBS strengths to set the threshold dose
  – Maximum repressor/antirepressor concentration will not change, only replacement rate

\( \gamma \) radiation
Final Dosimeter Construct

- Readable in most lighting conditions
- Output could be changed for different applications
## Progress

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Acknowledgements

Project ad

Drs. Ming Tien, Darryl Farber, William Hancock

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