Engineering thermostable regulators for inducible gene expression in thermophiles

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Engineering thermostable regulators for inducible gene expression in thermophiles

Connor Joyce, Guo Wu, Dr. Kang Wu
Geobacillus

Understanding thermophiles and their usefulness in industry

Inducible expression

Controlling gene expression

Why it matters

Why we want a thermostable LacI

Genetic engineering

The work I completed in lab and my results
Gram positive rod shaped bacteria

Thermophile - growth range higher than 40°C
  - Mesophiles grow below 40°C
  - Heat stable proteins and DNA contribute to its ability to survive at high temperatures
  - Subject to scientific inquiry

Solvent resistance capabilities - important for industry
  - Host cell for biofuel production
    - Can ferment C5 and C6 sugars into lactic acid, ethanol, formate, and acetic acid
BACTERIAL STRAIN EDITING

A
Resource Generation and Enrichment

B
Strain Development and Resource Refinement

Screening for promising algal strains

Pathway prediction and target selection

Scale up and product development

Directed or semi directed evolution of engineered strains

Condition specific omics data and constraint based model

Interactomic & fluxomic studies

High quality genome scale metabolic reconstruction

 Mathematical modelling and gap filling

Genome scale metabolic model

Oomics data generation and mining

Regulatory elements and gene regulation

Cas9/Cpf1 gene editing

BACTERIAL STRAIN EDITING
Inducible expression

- Promoter
- Repressor
- Gene

RNA polymerase
INDUCIBLE EXPRESSION

RNA polymerase

promoter

repressor

gene
INDUCIBLE EXPRESSION

- Protein
  - Translation
  - mRNA
  - RNA polymerase
  - Promoter
  - Gene
  - Repressor
WHY WE NEED INDUCTIBLE EXPRESSION

DESIRED GENE EXPRESSION

- Overexpression will flood the cell with protein, directing too much energy away from other metabolic processes to keep the cells alive.
- Underexpression will decrease yield.
- Well established tool in *E. coli* especially with the *lac* operon.
RNA polymerase

The Lac operon

The lac operator in the presence of lactose and the lac operator in the presence of no lactose

The lac operon in the presence of lactose and the lac operator in the presence of no lactose
RESULTS AND EXPERIMENTS
**DESIGN**

Mutant LacI expectations:
- Geobacillus + lactose→ expect fluorescence
- Geobacillus with no lactose→ expect no fluorescence

Wild type expectation:
- can’t bind to promoter- always on
- can’t bind to lactose- always off
- can’t bind to lactose or promoter- always on

Transform into Geobacillus
• Gibson assembly—used to join to pieces of DNA together and can be used to induce a mutation at the binding site
• Insert DNA into circular vector
  • Restriction enzymes—cut the DNA at specific nucleotide sequences
  • Ligase—reform the phosphodiester bond between two broken strands of DNA
• Used E.coli as initial hosts for plasmid production
Design

PED001

LacI

promoter

gfp

Restriction enzyme

PCR

Gibson assembly

Restriction enzyme

pnw33N

Restriction enzyme

Bacteria cell

Transformation

ligase

Transformation
**RESULTS**

Gibson assembly - DNA has joined together to create the three mutant LacI's

Transformation results: colony growth indicates successful transformation of plasmid
COLONY PCR

Transformation Experiment → Culturing of Transformed colonies → PCR reaction Preparation → Results

Finding the concentration in each step and the concentration of the enzymes for each step is critical and decreasing the concentration can increase yield.

Performed gel recovery to better isolate the correct size DNA.

Recreated the PCR templates used for gibson assembly.
Design

PED001

LacI

gfp

Restriction enzyme

PCR

Gibson assembly

ligase

Transformation

Bacteria cell

pnw33N

Restriction enzyme

Restriction enzyme
Decrease concentration of double digestion reactants further, as the yield over time for restriction enzymes decreases. Or, new enzymes can be purchased

If successfully transformed and colony PCR yields successful results, can transform into geobacillus.

Will need to perform sequencing to ensure proper gene sequence

The geobacillus can be given lactose, and green fluorescence can be measured to determine the thermostability of the lacI repressor protein in each of the three mutations compared to the wild type.
THANK YOU

Are there any questions?

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Emerging field of fuel production to reduce dependence on fossil fuels.

- Uses lignocellulosic feedstock material for ethanol production
- Uses triglyceride rich vegetable oils for biodiesel production
- In E. coli, bioethanol production has reached 40-55g/L
- Pretreatment of the feedstock, isolating the cellulose, hemicellulose, and lignin uses autohydrolysis or steam explosion
- Biofuel production hampered by cooling requirements and contamination with other bacteria strains.
- Using heat resistance bacteria, the cooling requirements and contamination problems can be resolved
Structural analog of lactose

Can't be metabolised- doesn't interfere with metabolism of plant material for biofuel production

Can still function as an activator molecule