How does environmental stress affect the growth of E. coli cells?

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Electrophiles and Oxidants

- Electrophiles are organic species that can accept electron pairs such as cations, alkyl halides, acyl halides and carbonyls.

![Example Electrophile](image)

- Oxidants are species that remove electrons from other substances.

- Both electrophiles and oxidants are reactive species that can react with proteins, thereby effecting signaling pathways in the cell.
  - In high concentrations they can result in cell stress or death.
  - In low concentrations they may be beneficial to the cell.
Targetable Reactive Electrophiles and Oxidants (T-REX)

- The T-REX approach allows us to deliver specific electrophilic signals or oxidant to a protein of interest, eliciting very specific responses rather than multipathway responses.

http://www.fasebj.org/content/29/1_Supplement/570.1/embed/graphic-1.gif
Application of T-REX to Evaluate HNE Effect on *E.coli* Growth

**Goal:** Construction of plasmids to express mRFPHalo and eGFPHalo in *E. coli*

- mRFP and eGFP are utilized to distinguish two populations of *E. coli*.
- The Halo domain is used to covalently attach the T-REX compound to the fusion protein.
- This system allows us to deliver low concentrations of HNE to a distinct population of *E.coli* and evaluate its effect on neighboring populations.
DNA Cloning Process

Step 1: Gene Amplification

Step 2: Extension of Megaprimers

Transformation

Step 3: Vector Digestion and Gene Insertion

Colony PCR
Step 1 and 2

mRFP ~678bp
eGFP ~720bp
Halo ~891bp
Step 2 PCR: Extension of Megaprimers for pet28a

The ends of both genes are extended with primers that anneal to the destination vector: pet28a.
Step 3: Empty pet28a Digestion and Gene Insertion

- Vector Digestion: Using the restriction enzyme EcoRI to cut out a region of the vector in order to insert our gene.
- Insertion of genes
Transformation, Colony PCR and Sequencing

- This step allows us to verify the presence of our genes and the base pairs.
Expression of mRFPHalo and eGFPHalo

No Induction

Induction
Synopsis

**Goal:** Construction of plasmids to express mRFPHalo and eGFPHalo in *E. coli*

- Cloning of genes
- Using fluorescent technology for visualization
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