Probiotic Sweetener, controlled by a pseudo-AND gate
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Abstract
We aim to use synthetic biology to engineer pro-biotic lactic acid bacteria used in the production of dairy products like yogurt, buttermilk and curds, to produce Maltol, a heat and pH stable sweetening proton. We plan to use the decreasing pH during curdling and the addition of milk as the two inputs, and the CRE gene in combination with loxP sites as an AND gate. In addition, we have used the unique functionality of Cre to create a system that is self-excision and will return the GMO to wild-type once the production is complete. We have managed to identify and characterise a pII-sensitive promoter, P70, and have abstracted SP101mut2 and CRE to be used in our system.

Concept
To conceptualize and synthesise a regulatory mechanism that functions as a pseudo-AND gate in response to two inputs.
Our pseudo-AND system is based on the gene system known as CRE-lox. We came across the CRE-Lox system, originally discovered in morphogenesis cells, but very effectively transferred to bacterial systems as a recombinase. Specifically, Cre-Lox recombination is a special type of site-specific recombination, which involves the targeting of a specific sequence of DNA and splicing it in the help of an enzyme called Cre recombinase.

Construct Design

NICR
The basic nuclear regulatory system is equivalent under the presence of signal II. Signal II is a protein that is often used or encoded for when the culture media is at lower pH levels. The system is found in a reaction with CreloX site.

SP101mut2
The output log is that which is achieved from the gene reactor at P70 and is an optimized version of the previously found SP101. The system is used under conditions of a lower pH at which the reporter log is an output.

Validation
CRE
To test the desired behavior of the CRE system, a plate based experiment is setup with the following construct and two reporters. On the transformation plate, different colonies will express the reporters based on the activity of the CRE within them. If the CRE functions within a particular cell, none of its future generations will carry the excised DNA fragment, therefore reporter 2 is expressed. Now, if CRE doesn’t function onto a certain generation, reporter 1 is expressed and the terminator stops the expression of reporter 2. If the reporters can be visualised independently then it may be possible to identify the point at which excision occurred.

References
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