Probiotic Sweetner, controlled by a pseudo-AND gate

Team: IIT_Madras

iGEM 2010 Jamboree

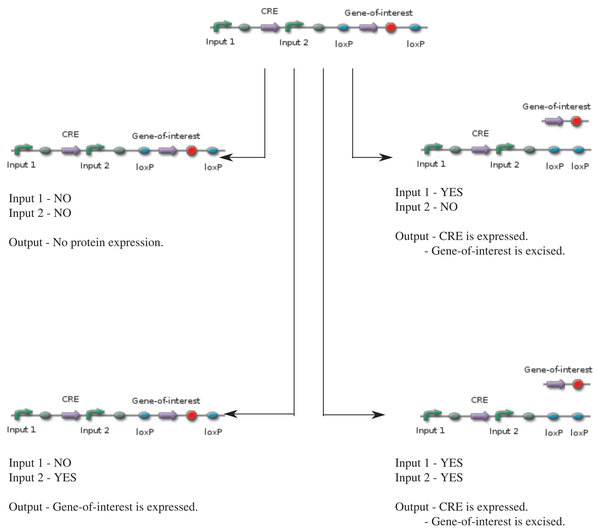
Abstract

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 Monellin, a heat and pH stable sweetening protein. We plan to use the decreasing pH during curdling and the addition of nicin 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-excising and will return the GMO to 'wild-type' once the production is complete. We have managed to identify and characterise a pH-sensitive promoter, P170 and have abstracted SP310mut2 and CRE to be used in our system.

Concept

To conceptualise 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 mammalian 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 with the help of an enzyme called Cre recombinase.



It can be seen that only one of the combinations of inputs give rise to an output, simulating a pseudo-AND gate. It is important to note that the Gene-of-interest is excised permanently, returning the cell (and its daughters) to 'wild-type'.

References

Morris J A, et al; Purification of monellin, the sweet principle of D.cumminsii, Biochem Biophys Acta (1972), vol 261:114

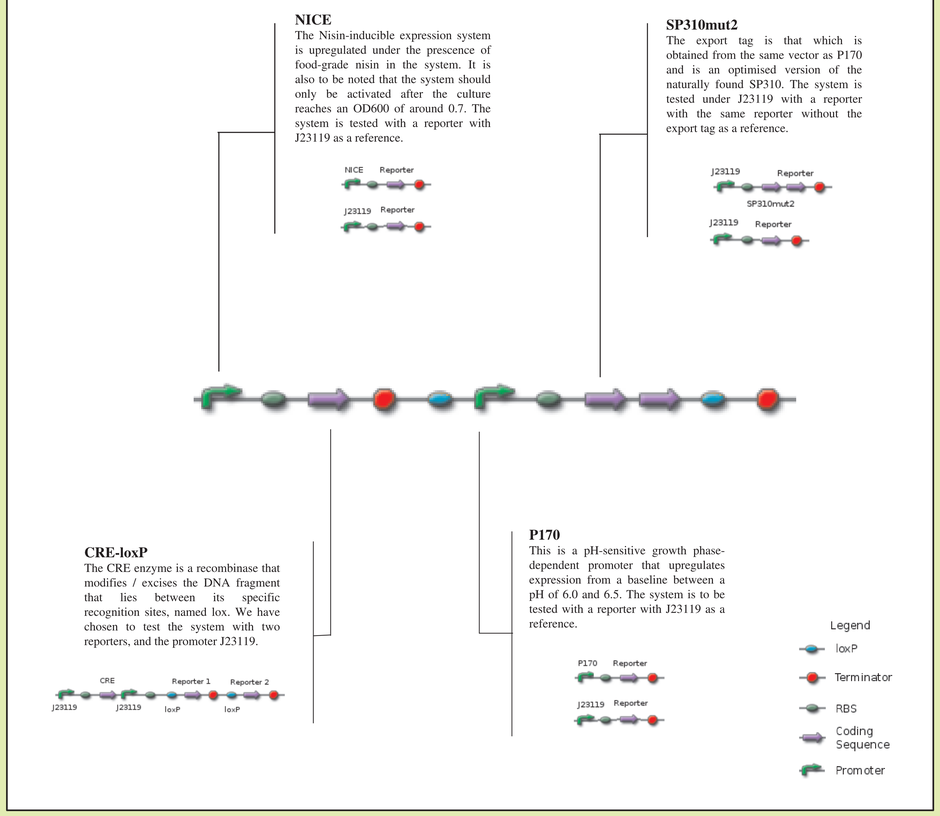
Madsen S M, et al; Molecular characterisation of the pH-inducible and growth phase-dependent promoter P170 of L.lactis; Molecular Microbiology (1999), vol 32, pg 75 - 87

Ravn P, et al; Optimisation of signal peptide SP310 for heterologous protein production in L.lactis; Microbiology (2003), vol 149, pg 2193 - 2201

Pfeifer A, et al; Delivery of the Cre recombinase by a self-deleting lentiviral vector: efficient gene targetting in vivo.

For further information and contact details of the IIT_Madras team, please visit our wiki at http://2010.igem.org/Team:IIT_Madras.

Construct Design



Our Team

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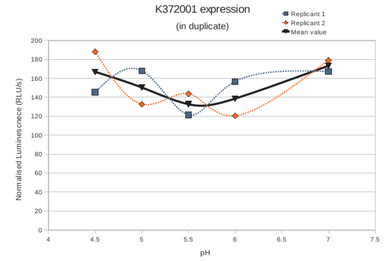
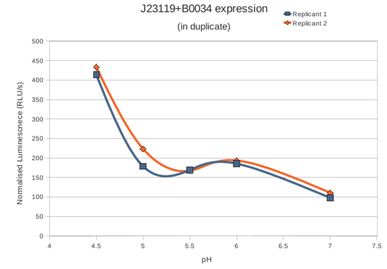
Results

P170

The testing of P170 was done at 5 different pH (4.5, 5, 5.5, 6, 7) with Firefly luciferase from pGL3Basic as the reporter. The data, given in Relative Luminescence Units/sec was normalised with OD600 and plotted against pH. The following trend was observed in the case of expression under P170.



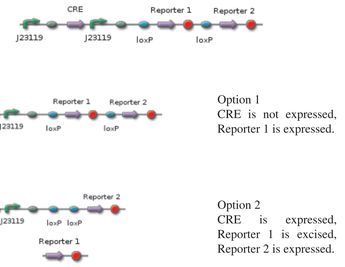
To our surprise, we also discovered that the constitutive promoter, which we used as a reference promoter showed up-regulation at low pH (4.5 – 5) indicating that it might be under the influence of a pH-based stress factor.



Validation

CRE

To test the expected 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 upto 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 maybe possible to identify the point at which excision occurred.



SP310mut2

The characterisation for the export tag will also be done in pGL3Basic with Firefly luciferase as a reporter. Samples will be taken over time and the luciferase will be assayed for both before and after cell lysis, to estimate export efficiency. Luminescence measurements of the luciferase were made in conjunction with added luciferin. The colonies are ready and the experiment will be conducted once we return from the Jamboree.

Our Sponsors

We also acknowledge the help of: