The Chinese University of Hong Kong

LSCI3000 Synthetic Biology Workshop

iGEM Idea Proposal

CaKit

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0 Abstract

The major allergen in milk is casein. Casein can interact with *Salmonella typhimurium* to shield it from acidic solutions and slow its decay rate. *Bacillus cereus* of RC6 strain can digest casein into antimicrobial peptides (AMPs) to give itself a competitive advantage over other microbial species. Regarding these two rationales, one can measure the light emitted by the bacteria transformed with bioluminescence proteins to estimate the casein concentration in a given solution compared to a negative control, hence allowing the detection of milk product content in foods or drinks.

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1 Background

1.1 Context

Around 2.6% of the population globally is allergic to milk and milk products (Motala & Fiocchi, 2011), with a fast-growing trend (Hochwallner, Schulmeister, Swoboda, Spitzauer, & Valenta, 2013). It is the most prevalent kind of food allergy in the world. The allergen in milk mostly comes from its protein content, with casein accounting for 80% of the total protein content (Hochwallner, Schulmeister, Swoboda, Spitzauer, & Valenta, 2013). Despite its allergy-causing tendencies, milk and its subsidiary proteins are not always classified as allergens in many countries if they are used as an additive. Hence, those who are allergic to milk often have to be very careful when choosing foods, and even then, they might not be able to entirely avoid milk products if they are imported from a country with the abovementioned regulations or travelling.

1.2 Specific Aims

The aim of this project is to provide a way for those who are allergic to milk to test if the food they are eating contains milk content without having to send it to a lab or have lab equipment on hand. The product aims to be quick and easy-to-use and ideally portable.

2 Design of Biobricks

2.1 Approach 1: Decay Rate

2.1.1 General Rationale

This approach of detecting casein leverages the protective effect casein has towards *Salmonella typhimurium* in an environment with lactic acid. According to Rubin (1985), for a 1.42% lactic acid solution, at just 4 per cent casein concentration, which amounts to roughly a 20 times dilution of regular milk, there is already a 13 minutes per log reduction in cell number. In his paper, he concluded that casein has a non-negligible effect on *ST* survival and further stated that the effect is more significant as pH increases. We can see that the decay rate reduction of *ST* is an effective indicator for casein concentration.

As for the decay rate measurement, I propose utilising bioluminescence. The basic principle of this method would therefore consist of transforming a bioluminescence gene (for example, GFP) into *ST*, then incubating it until the bioluminescence is at a standardised level and observable with a light measuring device (for instance, a flux meter), and finally mix in the lactic acid and casein solution. The decay rate of *ST* should be measurable with the flux meter as a rate of dimming of the luminescence level. A negative control without any casein would

also be concurrently run for comparison. In practice, the casein would come from food dissolved in water and act as the independent variable.

2.1.2 Required Biobricks

For this approach, any bioluminescence gene and plasmid should function as intended. For familiarity, I propose using GFP (green fluorescence protein) as the bioluminescence protein, pRSFDuet-1 as the plasmid, and *Bam*HI and *Hind*III as the restriction enzymes.

2.2 Approach 2: Competitive Advantage

2.2.1 General Rationale

This approach to detecting casein utilises the mechanism of biological competition. Within a confined environment, the species with a higher competitive advantage always have a more dominant representation by biomass. *Bacillus cereus* of RC6 strain is shown to be able to produce caseinase to degrade casein to generate antimicrobial peptides (AMPs), which can inhibit the growth of competitors in the same environment, giving *Bc*RC6 colonial advantages towards the ecosystem (Ouertani, et al., 2018). It should logically follow that given a casein-rich polymicrobial ecosystem, *Bc*RC6 should thrive over the other species, while in a regular casein-deficient ecosystem, the species should be expected to grow at their natural rates. Therefore, I propose that the biomass representation of *Bc*RC6 in a polymicrobial setting can indicate the concentration of casein.

For *Bc*RC6's competitor, I propose using a fast-growing species that cannot normally digest casein. In a casein-deficient environment, the competitor should be the dominant species in the sample due to its faster growth rate; in a casein-rich environment, *Bc*RC6 is expected to have an advantage due to being able to digest casein into AMPs as well as use it as an additional food source. The specific species of the competitor need not be too specific, but it must meet the criteria of not being able to digest casein, faster-growing than *Bc*RC6 but ideally not too fast, and not producing any antibiotics or toxins on its own. Further research might be required as I don't yet have a candidate for this competitor.

In terms of detection, I propose that bioluminescence can be used again. However, since we are concerned with two different species, I believe having them luminesce in two different colours could help better the measurement accuracy, seeing that one more additional variable can be measured. By computing the relative brightness of *Bc*RC6's luminescence and the competitor's, a casein concentration can be estimated using a casein-deficient culture solution as a negative control.

2.2.2 Required Biobricks

Similar to the first proposed approach, the bioluminescence protein and plasmid can vary, although the luminescence colour should be far apart in terms of wavelengths for ease of differentiation. Once again, for familiarity, *Bc*RC6 can be transformed using GFP (green fluorescence protein) as the bioluminescence protein, pRSFDuet-1 as the plasmid, and *Bam*HI and *Hind*III as the restriction enzymes. The competitor can be transformed using RFP (red fluorescence protein) as the bioluminescence protein, pBbe8k as the plasmid, and *Eco*RI and *Xho*I as the restriction enzymes.

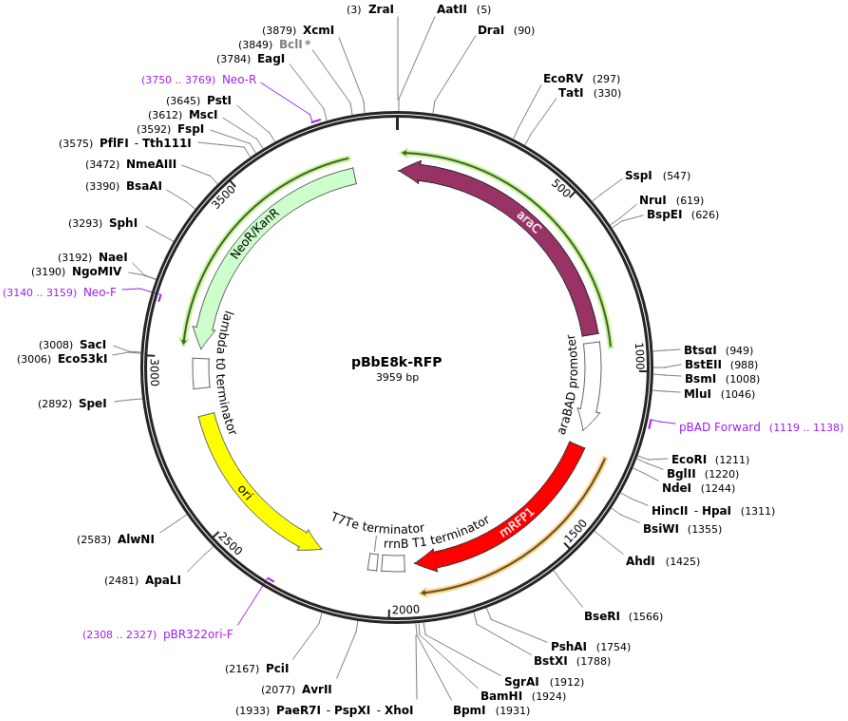

Figure 1 - pBbE8k-RFP

3 Applications and Anticipated Results

The final product should be a lightweight, portable test kit requiring only water to activate. Users should be able to use this test kit on the go, for example, when buying street food from vendors during vacation.

The major limiting factor is the speed of this kit. With enough research and calibration of the ideal pH and concentrations, the kit is anticipated to be able to perform the test within 5 minutes.

4 Discussion and Human Practice

This idea is somewhat extendable. Given the correct bacteria and bioluminescence proteins, the mechanism described in this proposal should be able to also work with other types of allergens. Considering that the current method for testing for allergens are predominantly using antibodies harvested from animal cells, this proposed method could potentially speed

up not only the testing speed by eliminating having to send samples to a dedicated lab but also the production of the test kits and hence should make allergen testing very affordable. In the long term, allergen testing could help decrease the demand for allergy medicines, such as EpiPens, by decreasing accidental allergen ingestion.

5 References

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