

CoralHue® Fluo-chase Kit Revamped

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Introduction

The CoralHue® Fluo-chase Kit was converted to work in bacteria. The advantages of this different platform are usability and cost to smaller research labs. This conversion allows for the creation of a protein-protein binding assay that can be used to screen natural products with only a fluorometer. Protein purification should not be required. This type of assay does not exist at UAH and would greatly increase the synergy of the LSB and third floor labs. In the realm of looking for a needle in a haystack, this assay would make the task much less daunting.

Basis

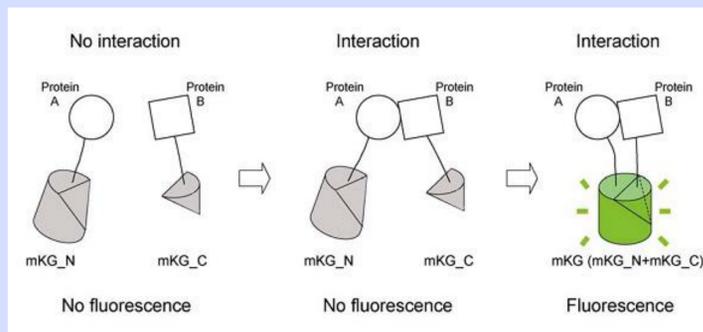


Figure1- Basic premise of the research. [1]

Background

Fluorescence is found throughout nature. A very interesting natural protein was found that fluoresced. The protein was able to be split into two pieces that would not fluoresce unless combined. The pieces would not bind together, so proximity was required by other means. Finally, there would not be fluorescence due to free floating interactions of the pieces in solution. The combination of all these characteristics allows the construction of binding assays. This was commercialized by Amalgaam and put in a mammalian plasmid.

Basics

The goal of this research is to find a natural product that can be used to inhibit specific protein-protein interactions. In order to do this efficiently, this project will attempt to utilize very small amounts of protein and use a sensitive method of screening. This protein assay will be ideal in that it will have an extremely low possibility of producing false positives.

Original Plan

The original plan for the project was to simply use mammalian plasmid in bacterial cells. The non-promoted growth of the fluorescing protein was hoped to be enough to work with the extreme sensitivity of the fluorometer. There was a pair of control proteins that were included in the kit. These control proteins were used for an attempt at proof of concept. They were grown up in BL21(DE3) and induced.

Initial Results

After several attempts, the mammalian plasmid was successfully transformed into bacterial cells. Everything went smoothly up until testing of the control protein. Initial results seemed perfect, but it was found that it was a false positive. There was not enough protein expressed in the lysate to cause be detected by the fluorometer. This meant that the original plan needed to be modified to convert the mammalian plasmid into a bacterial plasmid.

Modifications

Initial hopes were not achieved, so modifications needed to be made. An appropriate promoter region needed to be added to the plasmid to ensure that the bacteria would produce the needed protein. This was done by cutting and ligating the DNA into pET 28b vector. This would allow the production of the protein when the bacteria is induced.

Successes

As of now, successful transformation of the DNA was seen in the appropriate vector. The plasmid has been sent off to be sequenced to determine the official success of the steps.

To Come

If the sequencing shows correct ligation, the project will move back into protein phase. This should officially be able to prove proof of concept. Data similar to the data shown below would be what should be observed. The next step is attaching it to a protein and testing it. Successful attachment to a protein desired for testing could be the end of the project. Other projects, however, can use this assay to screen for natural products.

Perfect Data

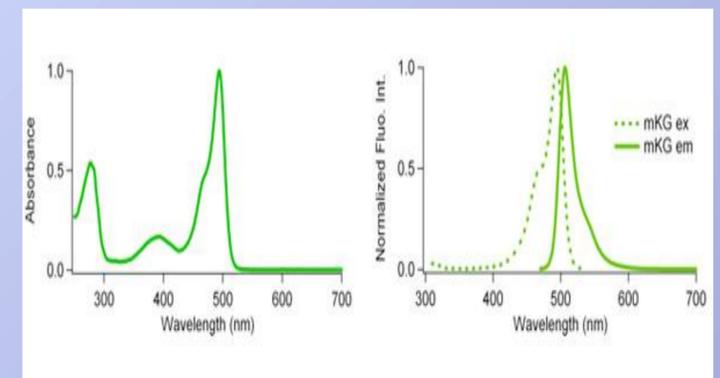


Figure1-Example of what the fluorometer data should look like. [2]

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Sources

[1] Found in the instruction material that came with the Coral Hue Chase Kit.

[2] From the company website. Accessed on 9/6/11. http://www.amalgaam.co.jp/products/advanced/fluo_chase_kit.html