CheckMate: A Rapid Yeast Mating Type Detector

Rose-Hulman Institute of Technology

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Abstract

Easily manipulated genetics make the yeast Saccharomyces cerevisiae a versatile and widely used model eukaryote. To progress, researchers must often determine the mating type of haploid strains, which typically takes days. The goal of our project is to reduce that time to hours. So we designed a novel promoter harboring Ste12 and LexA binding sequences and placed it upstream of an ORF encoding a red fluorescent reporter fused to LexA binding and VP64 activator domains. We propose that Ste12, activated in the pheromone response pathway, will bind the hybrid promoter and induce expression of the fusion protein, which will amplify and maintain its own expression. Therefore, when mating pheromone receptors on a haploid harboring this latch-type circuit are bound and activated, the cell will fluoresce and function as a rapid mating type detector.

Design

The CheckMate system was designed to simplify and speed up a mundane and time-consuming task in yeast genetics research. The goal of the project is to reduce the time required to determine a haploid’s mating type from one or two days to about four hours.

Modeling

Two differential models of the system were produced. One case uses one or more independent binding sites and the other exhibits cooperativity with various binding sites. Two approaches were employed to analyze the system’s signal, a deterministic model and a stochastic model. The results of these models suggest that for a given amount of mating factor, a constant level of Ste12 is produced (below).

The models produced predict possible scenarios that would allow for success of the project. While we have not yet determined the veracity of the models experimentally, they predict that project success is dependent solely on the values of the parameters of the system. Specifically, the decay terms must be less than the production terms. For the project to be successful, either the first or second depiction above must hold true.

The figures below illustrate three possible solutions predicted by the mathematical model. The x-axis is a measure of the external mating pheromone concentration and the y-axis represents the amount of reporter protein. The first illustration depicts a system where once the signal passes a particular threshold the circuit is turned on and will continue to produce protein. The second depicts a system where there is a particular threshold of mating pheromone required to bring about a stable level of protein; if the signal falls below this level, protein production is transient and returns to zero. The third depicts a system in which there is no discontinuity in protein production, and signal is required for all reporter protein production.

References:


Human Practices

Synthetic Biology Board Game

With goal of education in mind, the RHIT team developed a board game that teaches its participants as they play it. The game is called BioPioneer: Synthetic Biology. Players try to solve major world problems by selecting one of five laboratories, each with a special laboratory technique. Every player has to meet the world problem’s requirements while obtaining the correct DNA sequence that will code for the solution to the problem. Players take turns hiring people, buying equipment, and producing papers to help grow the knowledge pool and capabilities of the lab. The game was designed to incorporate actual procedures, resources, and equipment that are used in synthetic biology, creating a realistic laboratory experience.

Children’s Museum Exhibit

The second feature of RHIT’s Human Practices approach is geared toward younger children. A museum exhibit for the local Children’s Museum was created with the goal of giving children exposure to the concepts and models of synthetic biology. The Design Table introduces the kids to the idea of putting together DNA sequences. The instructions on the table ask them to think of ways they would use synthetic biology if they could make cells “do anything.” The team hopes that this will inspire them to use creativity to explore the possibilities of synthetic biology. After creating their sequence, the children move on to the Lab Bench, where they explore four different pieces of lab equipment. The stations are a microscope, a centrifuge, media plates, and pipetting. The exhibit has received positive feedback from museum staff, who are excited to see an exhibit on such a cutting-edge science.

NTNU Collaboration

iGEM has had a tradition of collaboration between teams since its inception. Teams are encouraged to share their strengths with other teams, whether they are strengths in modeling, characterization, or other aspects of the competition. Often, teams will exchange parts for characterization or sequencing. This gives the teams a chance to get to know each other, as well as experience with a different project. International collaboration is also fairly common; there is a website where teams can post offers or requests for help. When our team posted a request for help with stochastic modeling, we got three different offers for assistance within several days. This is a fairly typical experience for iGEM teams.

The 2012 Rose-Hulman iGEM team collaborated with the team from the Norwegian University of Science and Technology (NTNU). They shipped us a promoter they had extracted from E. coli and requested that we test it to see if it worked, and if so, get any quantitative data we could about it. We tested their promoter but were unable to induce expression of a reporter protein. In return for our test work, they built a stochastic model simulating the kinase cascade portion of our process, which backed up several of the assumptions that our team made in our differential model.

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