The Yeast Generation Counter

ABSTRACT

In our universe the fourth dimension, time, is always present. On our planet, our small but important planet, there are seven billion people that get older every day, every hour and every minute. To understand the ageing process a bit better we turn to synthetic biology. For the purpose of determining the replicative age of Saccharomyces cerevisiae a signaling cascade was invented, using fluorescent proteins as marker and Cas9 to create a logical AND gate for the counting of cell cycles. The desired dynamics were visualized using a mathematical model. And this is how I colored your mother.

Background

The determination of the replicative age, ergo the number of cell divisions, is a time-consuming and inefficient process. For Saccharomyces cerevisiae this is done by counting the resulting budding scars by microscopy imaging.

In our, much easier, approach a specific fluorescent protein is expressed depending on the number of divisions a (mother) cell has been through. In combination with flow cytometric or microfluidic devices an efficient and fast determination and sorting of the generation distribution within a culture can be enabled.

The major idea is a logical AND gate with dCas9 and a gRNA molecule, which together activate the signal cascade for the fluorescence protein of the next cycle.

Constructs

Our concept consists of four constructs, each on a separate plasmid. Three of these constructs are activated by the cell cycle, whereas the fourth is activated by the dCas9-gRNA from the cell cycle before. For the first construct, ergo the first fluorescent protein and the first gRNA, a daughter cell specific promoter is being used. This ensures a reset for every new daughter cell.

The split of the gRNA from the mRNA for the fluorescent protein is carried out by the intronless cleavage activity of ribozymes.

The remaining gRNA is protected by Bbrt-caps on each end, which are cleaved off by Cas9.

As well as the expression of Cyc1, the expression of dCas9 is triggered by the cell cycle. In combination with the gRNA the transcription factor dCas9-V404 is formed. It triggers the expression of the fluorescent protein and gRNA of the next cycle.

Degradation-tags ensure the cleansing of every protein before the next cycle starts.

Modeling

In our mathematical model we tried to reproduce the ideal dynamics of the system species in Matlab. The model was realized by means of ordinary differential equations in which the variables correspond to the cellular species while the parameters represent actual biochemical constants.

Assumptions are made in order to make the model clear and intuitive. In addition to the two colors of our constructs in the lab a third color is present in the model, representing a count until generation three.

Methodology

By transforming plasmids containing the correct fragments colony PCR's were performed. The yeast cells were plated on selective media. Only the successful transformation of the plasmids with the amino acid markers enabled growth. To ensure that these plasmids contained the correct fragments colony PCR's were performed.

Since our constructs express fluorescence proteins, we observed our transformed cells with a fluorescence microscope. The pictures not only confirmed the successful transformation. The yellow fluorescence confirms the desired cleavage function of the ribozymes. Also, since only the daughter cells are fluorescent, the functioning of the degradation-tag for the fluorescent proteins was confirmed.

Results

In the beginning of July one of our team members and a member of Gothenburgs iGEM team of 2012 had a radio interview on the Swedish radio station P4 Göteborg. There they talked about the iGEM competition and synthetic biology in general.

As a start of the study year 2014/2015, we held a presentation about iGEM, synthetic biology and our team for the new students at our university to raise awareness about the topics and recruit new members for next year.

We also happily attended a weekend in Linköping for a Swedish iGEM meeting with the teams from Linköping and Uppsala, discussing about ethics and making new friends. An essay was also written on ethics in synthetic biology which can be read on our homepage.

Human Practices

In addition to the above, our team members went to the Swedish beach to promote the Swedish language.

The Team

Our team reflects the diversity of the iGEM competition, not only in one way. From the total amount of ten student members, five of us are not Swedish but come from different nations, including Brazil, India, Hungary, Italy and Germany. Additionally, we are interdisciplinary (bio-tech, mathematics, food science) and half of the team is at undergraduate level, giving everybody the unique opportunity to learn and help each other in many different ways.

Student members: Gustaf Edman (Team 2014) & Lina Persson (Team 2015) at the studio P4 Göteborg.

Supervisors: E. Fischer, D. Julisson, V. Suneer

Methodology

**Primer & PCR design**

**gBlock design**

**Digestion with restriction enzymes**

**Plasmid extraction**

**Transformation into S. cerevisiae**

**Intrinsic homologue recombinase activity**

**Colony PCR**

**Fluorescence Microscopy**

**Microscopy Image of assembly construct 2 yeast colony. Daughter cells show yellow fluorescence**

**Plating and screening**

**Figure 1** Schematic Illustration of the logical AND gate.

**Figure 2** Fluorescence Microscopy Image of assembly construct 2 yeast colony. Daughter cells show yellow fluorescence.

**Results**

**Signal cascade of the constructs, showing the cascade from transcription to degradation**

**Constructs**

**Human Practices**

**Methodology**

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