The Team

We are Team Chalmers-Gothenburg, consisting of 15 members from the rainy city of Gothenburg. With students of different backgrounds, we have managed to utilize each individual’s strengths to optimize our workflow throughout the summer.

The Concept

Bioreactors are used in the production of both food and medical substrates, but one problem with these reactors is the risk of contamination. The main goal of our project is to detect and eliminate contaminants in bioreactors, to save both time and material. To achieve this, two systems are implemented into Saccharomyces cerevisiae. The first system is a detection system which uses the pheromone pathway to detect contaminations. The second system is a DNA-repair system that will make it possible to eliminate the contamination by UV-irradiation without killing the yeast. In order to avoid spreading this yeast with increased resistance to nature, a high concern of ours, a safety switch has been implemented.

The Detection System

The detection system, is based on a modified pheromone pathway1, where the GPC-receptor is modified to be activated by pheromones from S. pombe (containment). The result is a signaling cascade that uses an amplification system to express RFP as the output signal.

The Safety Switch

The safety switch, constructed to prevent the yeast with increased resistance from spreading in nature, is based on the SUC2 gene regulation. This gene is important for yeast to be able to metabolize sugar dimers, i.e. sucrose, in the absence of glucose. A damaging protein3, Tpk2, is introduced which is expressed instead of SUC2 when glucose is absent. The expression is increased by a second promoter (pTEF1)4. Practically, this means the yeast will die outside the lab due to a lack of glucose.

Results

The DNA-repair system uses enzymes from the RecA system in D. radiodurans, an extremely UV-resistant bacterium2. These enzymes collaborate with DNA repair enzymes naturally present in yeast to repair double-stranded breaks in the DNA helix.

Achievements

- Constructed and integrated:
  - Two different safety switches, based on TPK2 overexpression or mRFP expression induced at low ATP levels, into CEN.PK2.
  - The system to detect the P-factor from S. pombe into CEN.PK2.
  - SS TPK2 shows reduced growth rate after OD-measurement but no change in viability compared to wild type.
  - Fluorescent measurements:
    - show that connection of pTEF1 to pSUC2 maintains the ATP repression mechanism of pSUC2, while achieving higher expression at low ATP levels.
    - Verified that the fusion GPC-receptor is functional
    - Amplified all parts of the DNA repair system, but obtained vector-only clones after Gibson and transformation into E.coli.

To the left: Modeling of the safety switch, with both pTEF1 and pSUC2 as promoters, in absence of glucose. To the right: Test of the expression values of the safety switch in S. cerevisiae (CEN.PK2) with RFP, in presence of glucose.

 References: