An Engineered Bacterium with Adjustable Timer and Counter Functions

Motivation

When fresh food displays in a supermarket, it is necessary to detect if bacteria contaminate food or not. However, it is hard to design an economical mechanism which can count the population of invading bacteria at real time and also can control the fresh food storage time.

To overcome this problem, our team designed a bacterial referee with several functions:

- **Timer**: Bacterial referee counts variable time duration
- **Counter**: Bacterial referee calculates the population of invading bacteria at real time.
- **Memory**: Bacterial referee memorizes the system that starts or not.
- **Self-annihilation**: Bacterial referee destroys itself by using cdtB killer gene after finished the task to avoid environmental pollution.

Input and Output Signals

The bacterial referee system works as an OR gate which integrates temporal and environmental signals. The outputs are fluorescent proteins.

The green fluorescence means that the referee system is turned on and the environment is safe, whereas yellow means the system is beginning to lose its freshness, and red warns us that there is bacterial contamination or the food has expired.

Timer

The working length can be determined by the concentration of lactose. A key feature of the bio-timer is the lag time function up to 8-20 hrs.

The timer has three stages:

1. **Standby stage**: timer not start yet, and the medium is colorless
   - When bacteria grow in lactose-free medium, the lacI promoter is not expressed and the medium is colorless.
2. **Lactose-accession phase**: the medium is green
   - When lactose is added in the medium, lactose binds with lacI repressor and releases the inhibition of lac promoter to activate transcription of GFP. The medium is becoming in green color at this stage.
3. **Lactose-consumption phase**: the medium is red
   - When added lactose is consumed by E. coli, the red fluorescent protein (RFP) starts to translate and GFP is degraded. The red color in the final step warns us that time’s up.

Simulation results of timer function:

The simulation data indicated the concentration of lactose determines timer’s working length. Low concentration of lactose decreases the intensity and duration of green fluorescence. After added lactose is consumed by E. coli, the RFP is translated to remind us that time’s up.

Concepts

**Durations**

- **TIMES UP**
- **START!**
- **BACTERIA INVASION**

**Stages**

- **Steady phase**
- **Lactose-accession phase**
- **Lactose-consumption phase**

**Memory**

The memory device records "lactose has been added" message in our bacterial referee system to maintain media colorless until the lactose is added. The basic idea of this memory system was first developed by tUcasen during the 2008 iGEM competition. Do you notice? There is no lactose in the steady stage and lactose-consumption phase, but their working DNA strands are different. This is the ability of the memory device.

Counter

The counter function can detect the bacteria population with LuxI/LuxR device. If external bacteria invade the system, the extra AHL produced by them will bind the LuxR protein to form the LuxR/AHL complex. This complex activates target promoter Pmuc, to transcribe the RFP to warn us the contamination.

Simulation results of counter function:

When external bacteria invade the system, they increase the production rate of LuxI protein (i.e., α_LuxI). Thus, the AHL/LuxR complex binds the Pmuc/AHL promoter to activate transcription of the downstream RFP. Therefore, RFP is produced quicker with higher concentration of invading bacteria.

New Idea

Our team designs a simple protocol to generate a promoter library. This promoter library with different transcripational strength can be built to tune the specific parameter values that model equations indicated.

Degenerated primers designed for PCR are used to generate mutations in promoter regions. The ~35 or ~50 sequences are amplified by degenerated primers with some random mutations (red star), then each library can represent many variations in promoter strength.

Applications

Bacterial referee can be applied in:

1. **Food preservation**
2. **Contact lenses**
3. **Trauma dressings**

Green fluorescence intensity of the mutated promoter PluxI. Mutated promoter activities are from the low to the high-stake (wild type: K145270). That means our protocol is practicable!