Pathway Commander: Metabolic Control via Thermo-induced Device

**Abstract**

Pathway Commander is a method that controls the flux through a biosynthesis pathway using thermo-induced devices. By this method, we can use culture temperature shifts to control the expression levels of a series of metabolic compounds at the precise times. We have implemented the Pathway Commander design in (1) Carotenoid synthesis Pathway, (2) Violacein biosynthesis pathway and (3) Iso-butanol synthesis pathway in E. coli. This circuit design utilizes a temperature controlled system that gives precision control over metabolic compound expression which amounts to optimized synthesis of a given compound or drug.

**Thermo-induced Device**

We found two thermo-induced devices and calculated their protein expression ability at the different temperature.

1) **37°C Induced RBS : BBa_K115002**

![Figure 1- 37°C induced device works as a RNA thermometer. The RBS (ribosome binding site) form a hairpin structure. When a temperature is reached, the hairpin unwinds permitting mRNA to be translated.]

![Figure 2- Green fluorescence intensity results of RNA thermometer. The flow cytometer's data shows the highest fluorescent protein expression at 37°C and 42°C. That proves the 37°C induced RBS has an appropriate function.]

![Figure 3- The relative translational activity of 37°C induced RBS BBa_K115002 at 25°C, 30°C, 37°C and 42°C were estimated by model equations.]

2) **42°C Induced CI promoter : BBa_K098995**

![Figure 4- Gene encoded for CI protein located downstream of a constitutive promoter. At temperature lower than 42°C, CI protein dimer binds to Pci and represses it. When the temperature is higher than 42°C, CI dimer degrades, Pci is turned on and expressed.]

**Carotenoid Pathway**

- Farnesyl diphosphate
- Geranylgeranyl diphosphate
- Phytoene
- β-carotene
- Lycopene
- Zeaxanthin

**30°C device**

**37°C device**

**42°C device**

![Figure 5- The overview of carotenoid pathway. Schematic diagram of expression control via temperature shift. This pathway explain how Farnesyl Diphosphate is catalyzed to Zeaxanthin by shifting temperatures. We can get three products using one circuit depending on culture temperature shift!]

**Violacein Pathway**

- vioA
- vioB
- vioC
- vioD
- Protodeoxyviolaceinic acid (PVA)
- Deoxychromoviridans

**30°C**

**42°C**

**37°C**

![Figure 7- Violacein pathway. We also can control the direction of pathway with branches. Intermediate PVA can turn into Deoxychromoviridans or Protoviolaceinic acid regulated by different temperatures.]

**Butanol Synthesis Pathway**

- Glucose
- Pynvate
- 2-Acetolactate
- Isobutyraldehyde
- 2,3-Dihydroxyisovalerate
- Iso-butyraldehyde

**37°C**

**30°C**

![Figure 9- The butanol synthesis pathway. Butanol Synthesis needs to be controlled because isobutyraldehyde and the end product, isobutanol are toxic to E. coli. When the final gene kviD is turned on, the accumulated intermediate would soon metabolize to isobutanol.]

**Figure 10- The low temperature release circuit design of butanol synthesis pathway. We clone the genes which can be translated into enzymes such as AlsS, Ikc, IivD, KivD, and assemble the genes into two circuits for producing butanol.**

**Figure 11- The GC results of the amount of isobutanol in the medium.**

**Figure 12- Comparison of isobutanol production under low-temperature released device or not. The Butanol production rate is higher in low-temperature released device. We successfully improve the production of isobutanol by low-temperature released device.**

**Achievements**

- Designed “Pathway Commander” method to control pathway.
- Controllable carotenoid synthesis pathway work as expected.
- Provide several novel biobricks can produce butanol!
- Controllable butanol synthesis pathway work as expected.
- Characterize thermo-induced devices.
- Correct point mutations of previous biobricks!