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CTCI Science and Technology Creativity Scholarship

Pathway Commander

Metabolic Control via Thermo-induced Device

NCTU DBT

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) Isobutanol 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

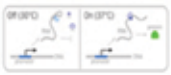

Figure 1- 37°C induced device works as a RNA thermometer. The RBS (ribosome binding site) form a hairpin structure. When a temperature is reached, the hairpin unwinds ,permitting mRNA to be translated.

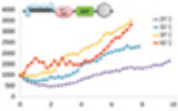

Figure 2- Green fluorescence intensity results of RNA thermometer. The flow cytometer's data shows the highest fluorescent protein expression at 37°C and 42°C. That proves the 37°C induced RBS has an appropriate function.

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

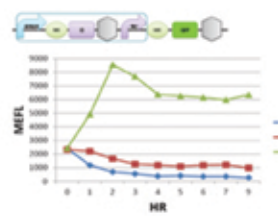

Figure 4- Gene encoded for CI protein located downstream of a constitutive promoter. At temperature lower than 42°C, CI protein dimer binds to P_{cI} and represses it. When the temperature is higher than 42°C, CI dimer degraces, P_{cI} is turned on and expressed.

Carotenoid Pathway

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!

Figure 6- The circuit design of Carotenoid Pathway. *crtE ,crtB ,crtI* are expressed at temperature lower then 37°C. *crtY* is expressed at temperature higher then 37°C. When the temperature rises to 42°C, CI protein is degraded and pCI is no longer inhibited , leading to *crtZ* expression.

Violacein Pathway

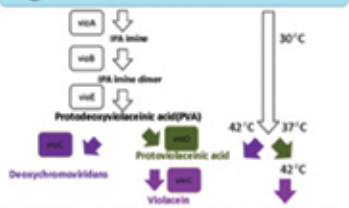

Figure 7- Violacein pathway . We also can control the direction of pathway with branches. Intermediate PVA can turn into Deoxychromovividans or Protoviolaceinic acid regulated by different temperatures.

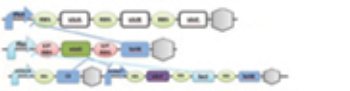

Figure 8- The circuit designs. When culture at 30°C,vio A, vioB ,vioE is expressed, thus we will turn left, vioC is expressed, the colorless product turns into dark purple. When shifting from 30°C to 37°C, pathway will turn right, resulting in vioD expression. PVA will turn dark green. Shifting from 37°C to 42°C will express vioC and produce Violacein.

Butanol Synthesis Pathway

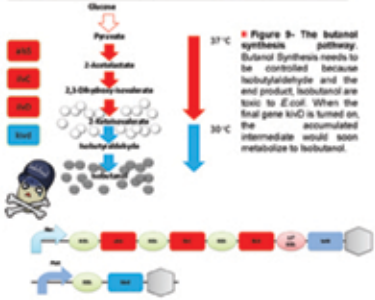

Figure 9- The butanol synthesis pathway. Butanol Synthesis needs to be controlled because Isobutyraldeidyde and the end product, Isobutanol are toxic to *E.coli*. When the final gene *kivD* is turned on, the accumulated intermediate would soon metabolize to Isobutanol.

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, IlvC, IlvD ,KivD*, and assemble the genes into two circuits for producing butanol.

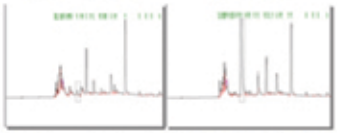

Figure 11- The GC results of the amount of isobutanol in the medium.

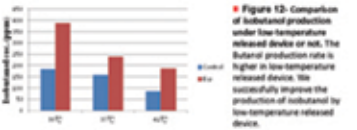

Figure 12- Comparison of isobutanol production under low-temperature released device or not. The Butanol production rate is higher in low-temperature released device. We successfully improve the production of isobutanol by low-temperature released device.

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