CrtEBIY BBa_K274200 Experience

Characterization by Peking iGEM Team 2010

We find that BBa_K274200 represented a significant leakage expression when exploited as a reporter gene and even bacteria bearing BBa_K274200 only could also represent significant color change, compared with the blank. We speculate that it's because of a putative promoter upstream, resulting in the leaky expression of CrtEBIY. In order to verify the speculation, we further characterize this biobrick. This biobrick was suffixed to the constitutive promoter BBa_J23103. Resulted new biobrick is shown in Figure 1.

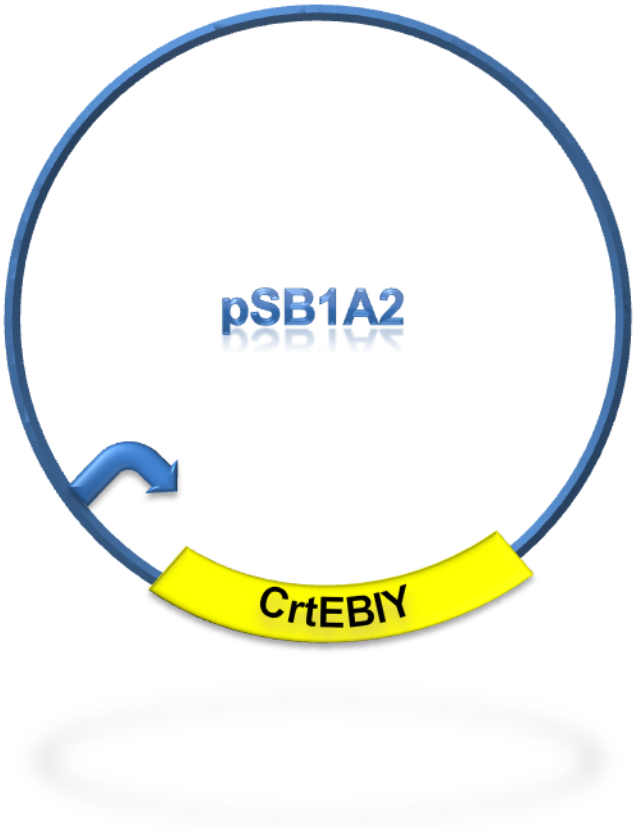
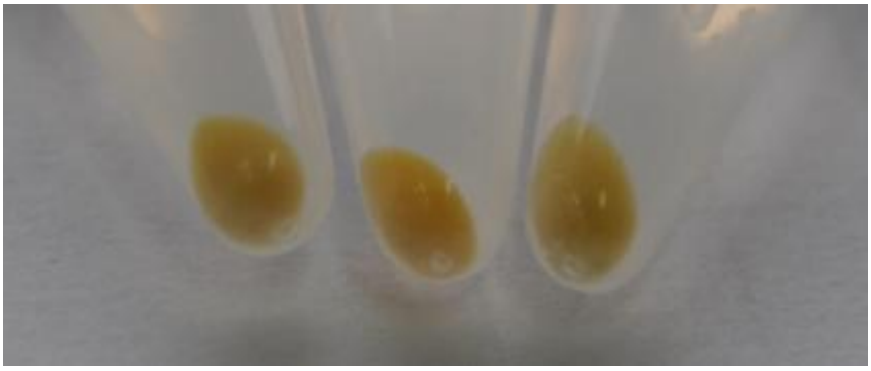


Figure 1. The biobrick we constructed to characterize biobrick BBa_K274100. It is Constitutive promoter BBa_J23103 driving CrtEBI.

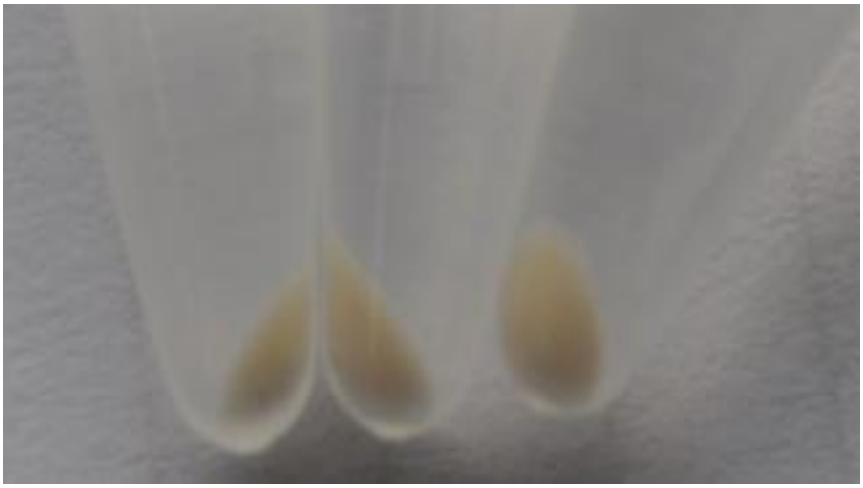
After the biobrick was constructed, it was transformed to JM109, BL21 (DE3) and DH5α, respectively. Then we compared their color with the corresponding strains that bear plasmid with only CrtEBIY as the insert.

After cultivated at 37℃ for 12 hours, as shown in Figure 8, bacteria bearing BBa_J23103-CrtEBIY and naked CrtEBIY represented a yellow color and blank vector appeared white (Fig 2).

Naked CrtEBIY



Constitutive promoter BBa_J23103-CrtEBIY



Blank

Figure 2 **Color contrast after 12 hours' cultivation (for each photo, from the left to the right are JM109, DH5α and BL21)**

From these pictures above, we can see that both strains bearing constitutive promoter-CrtEBIY and strains bearing naked CrtEBIY appeared yellow. This demonstrated that CrtEBIY has a serious leakage, and the cause of leakage is probably the same as CrtEBI. It is noteworthy that CrtEBIY pathway need one more enzyme (Lycopene Cyclase, produced by CrtY) than CrtEBI and the Km for Lycopene Cyclase is 1.8μM (Schnurr et al., 1996), so the leakage was more serious as expected.

We intended to use CrtEBI and CrtEBIY as our bioreporter at first. After our characterization, we decided to use CrtEBI for the serious leakage of CrtEBIY. However, we found a serious leakage when CrtEBI was combined with the promoter PmerT and PpbrA. Leakage was serious regardless of the prefixed promoter in our experiment. For this reason, we chose other reporter gene, such as LacZ alpha instead of CrtEBI.