Characterization Existed Parts

1. CrtEBI Characterization

Biobrick BBa_K274100 is the lycopene-producing device submitted by team Cambridge 2009. It is used to produce lycopene which is red pigment can be detected by the naked eye.

However, we find that BBa_K274100 represented a significant leakage expression when exploited as a reporter gene and even bacteria bearing BBa_K274100 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 CrtEBI. In order to verify the speculation, we further characterize this biobrick. This biobrick was suffixed to the constitutive promoter BBa_J23103. On the other hand, we did something fantastic: The biobrick was **suffixed** to the terminator BBa_B0015. Terminator upstream was expected to reduce the basal level of lycopene production, thus to verify our speculation. Resulted two new biobricks are shown in Figure 1.

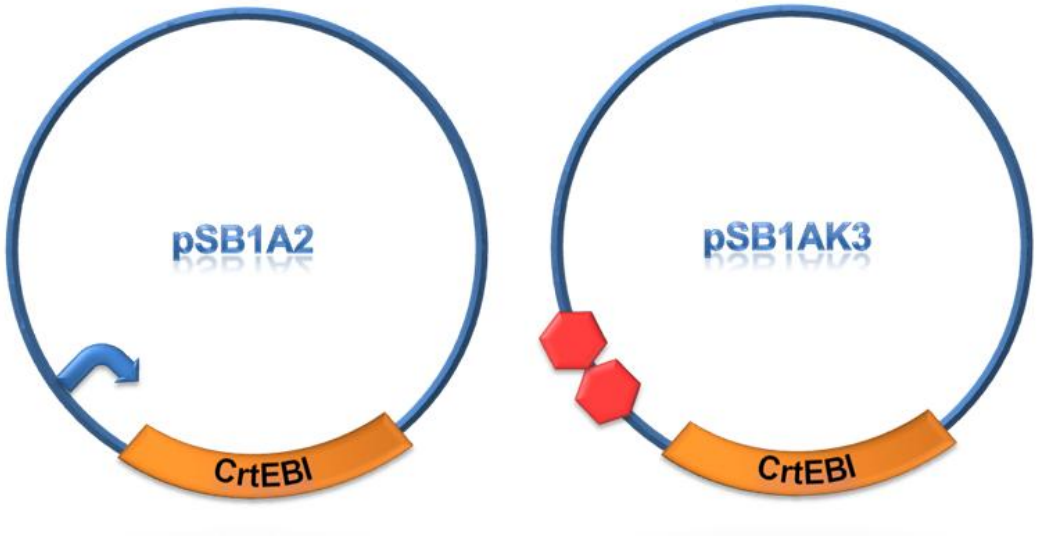
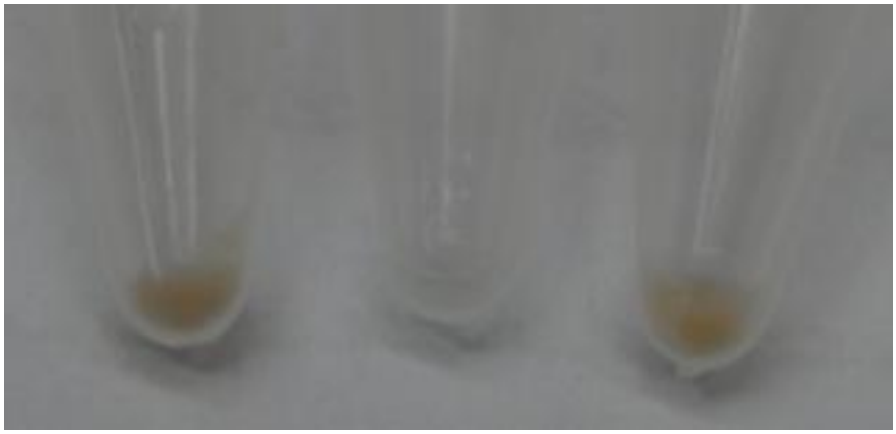


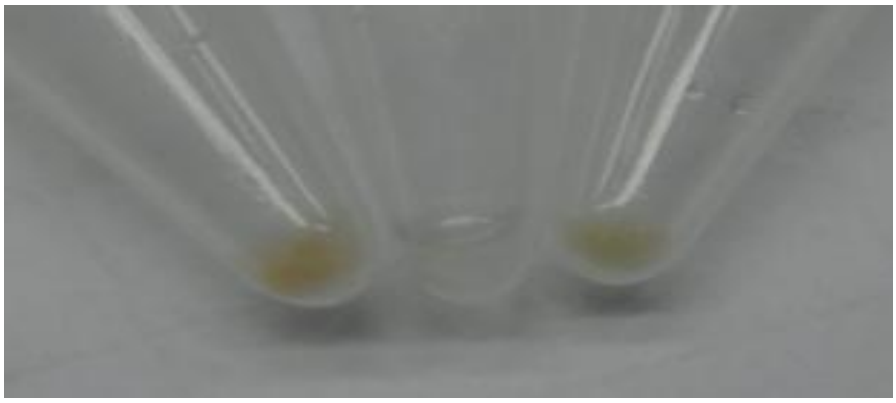
Figure 1 Biobricks we constructed to characterize biobrick BBa_K274100. The left construct denotes Constitutive promoter BBa_J23103-CrtEBI and on the right is an interesting biobrick—Terminator BBa_B0015-CrtEBI, namely a terminator was prefixed to CrtEBI, expected to reduce the leaky expression.

After the two biobricks was constructed, they were transformed to JM109, BL21 (DE3) and DH5α, respectively. Then we compared their color with the corresponding strains that bear plasmid with only CrtEBI as the insert, at different time intervals.

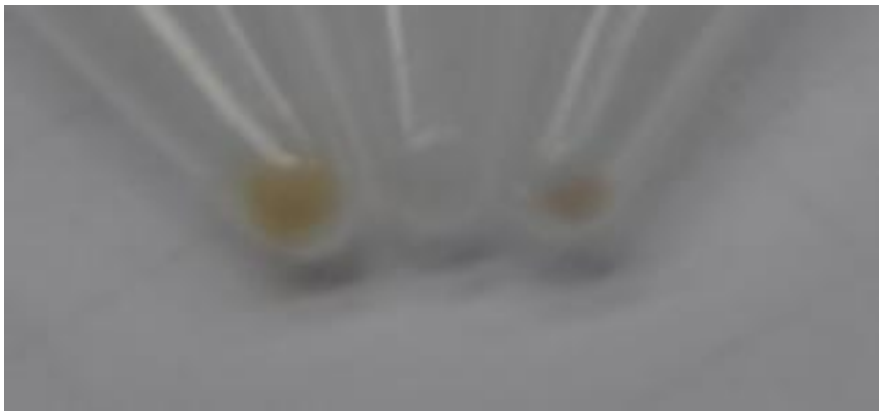
After cultivated at 37℃ for 9 hours, as shown in Figure 2, bacteria bearing BBa_J23103-CrtEBI and naked CrtEBI represented a light red color while bacteria bearing Terminator-CrtEBI and blank vector appeared white (Fig 2). We can't collect bacteria of strain BL21 (DE3) at this time because it was slow-growing in this experiment for some reason.



Constitutive promoter BBa_J23103-CrtEBI



Terminator BBa_B0015-CrtEBI



Naked CrtEBI

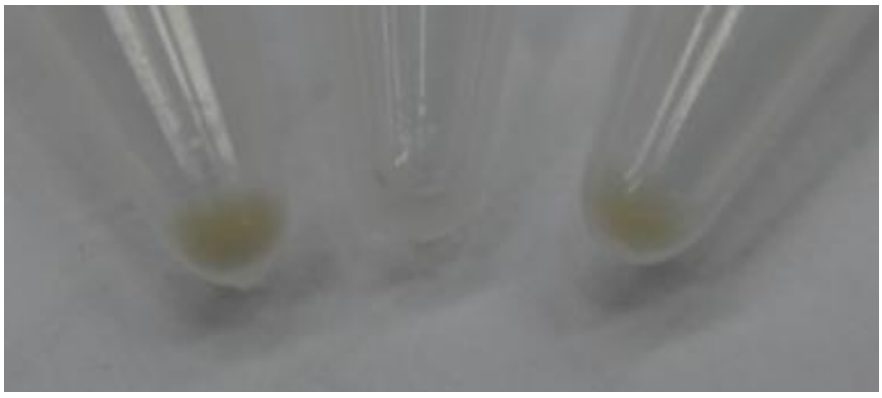


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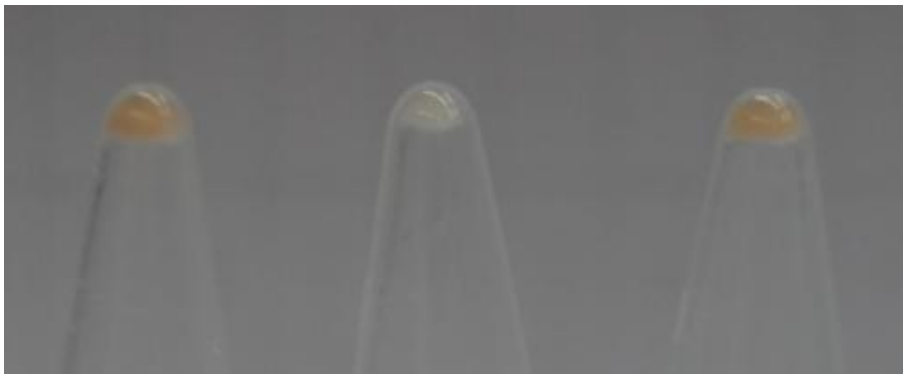
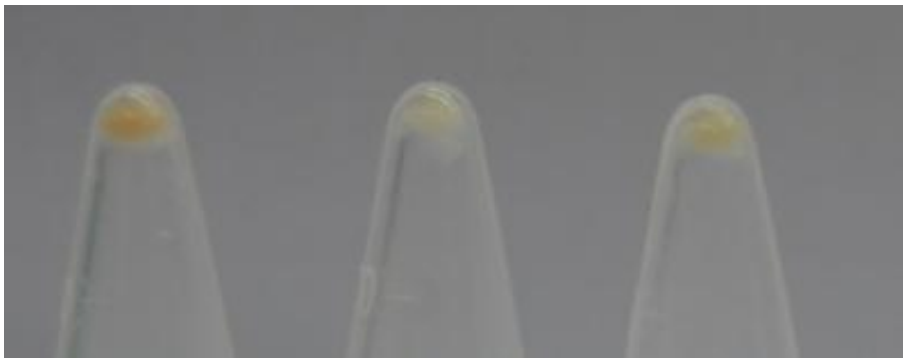
Fig. 2 Color contrast after 9 hours' cultivation (for each photo, from the left to the right are JM109, BL21 and DH5α).

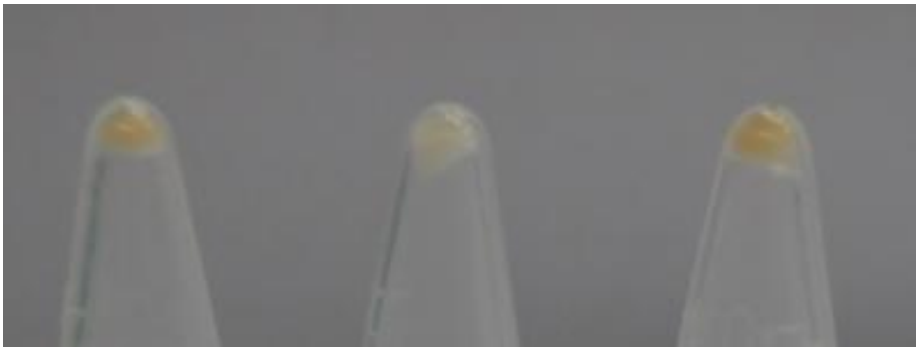
After 12 hours' incubation, strain BL21 (DE3) appeared white in all cases. Besides, constitutive promoter BBa_J23103-CrtEBI appeared red while CrtEBI still represented light red. The terminator-CrtEBI appeared light red in strain JM109 and still white in DH5α. The results are shown in Figure 3.



Constitutive promoter BBa_J23103-CrtEBI



Terminator BBa_B0015-CrtEBI



Naked CrtEBI

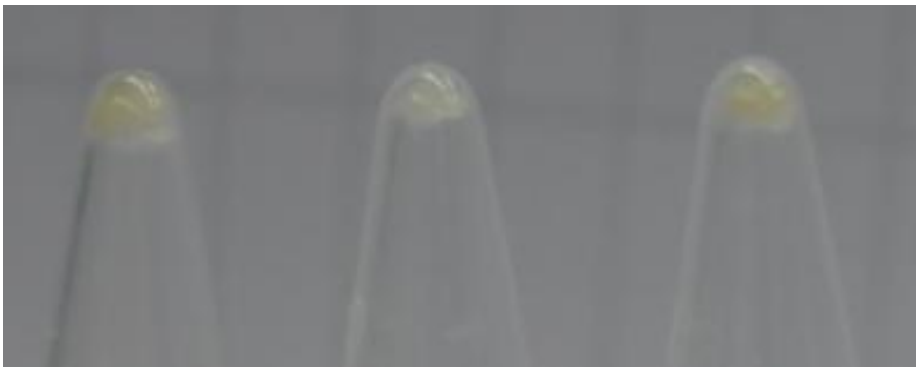


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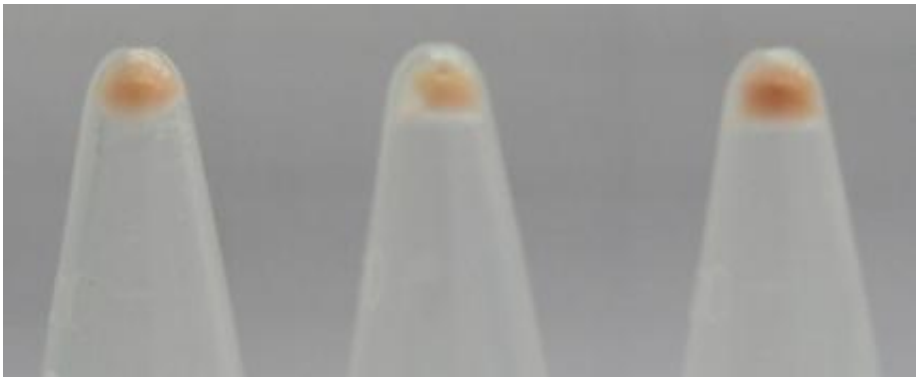
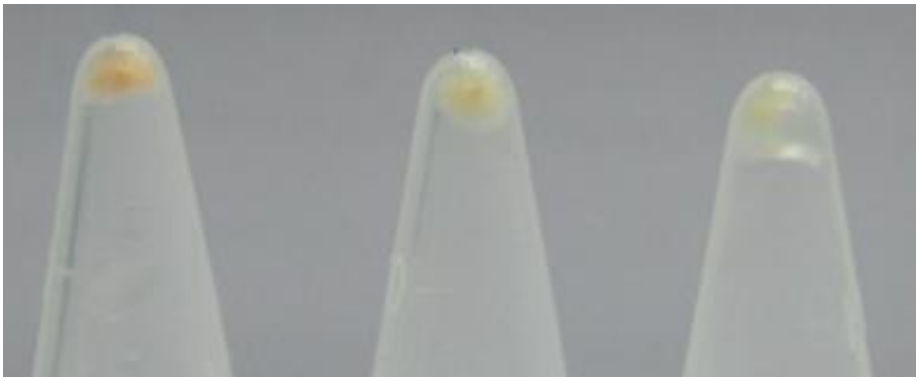
Fig 3. Color contrast after 12 hours' cultivation (for each photo, from the left to the right are JM109, BL21 and DH5α).

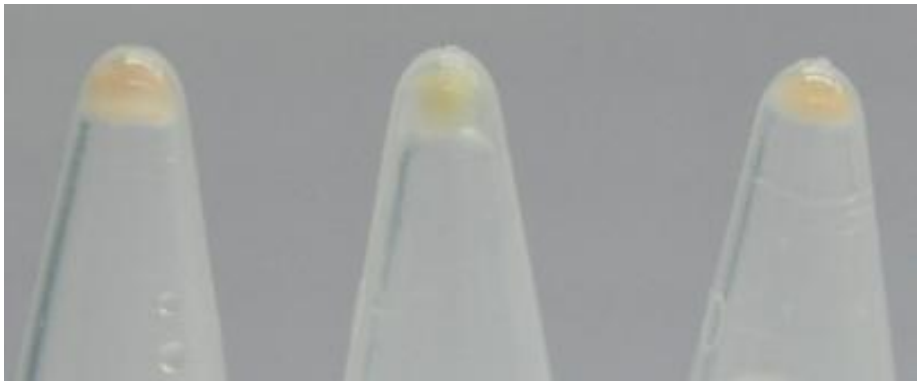
After 15 hours, as was shown in Figure 4, the situation was the same as 12 hours. The only difference was that strain BL21 (DE3) appeared red color. BL21 (DE3) constitutive promoter BBa_J23103-CrtEBI appeared red while naked CrtEBI appeared light red. BL21 (DE3) terminator-CrtEBI represented white like the blank.



Constitutive promoter BBa_J23103-CrtEBI



Terminator BBa_B0015-CrtEBI



Naked CrtEBI

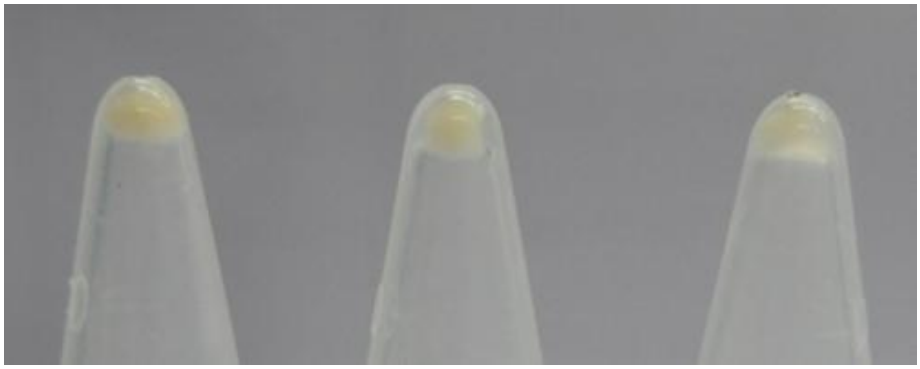


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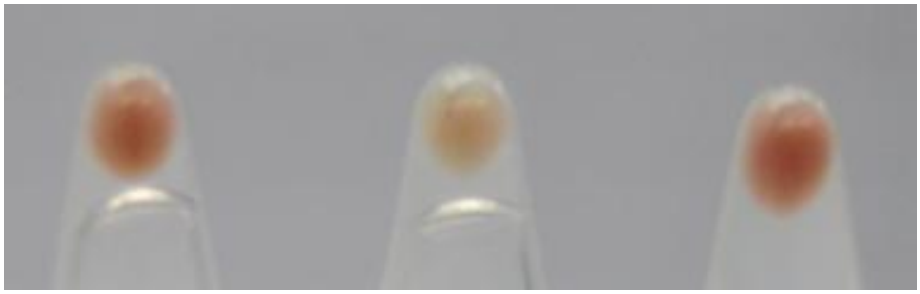
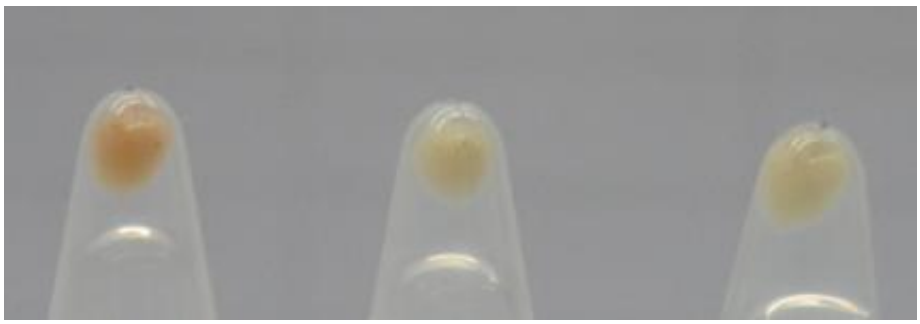
Fig 4. Color contrast after 15 hours' cultivation (for each photo, from the left to the right are JM109, BL21 and DH5α).

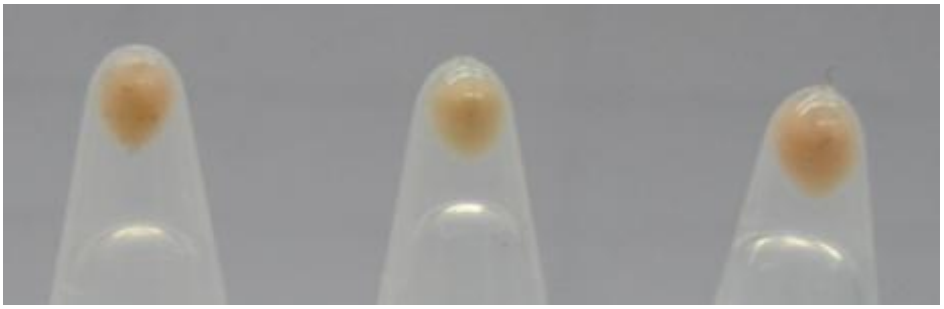
After 24 hours, the situation was the same as 15 hours as was shown in Figure 5.



Constitutive promoter BBa_J23103-CrtEBI



Terminator BBa_B0015-CrtEBI



Naked CrtEBI

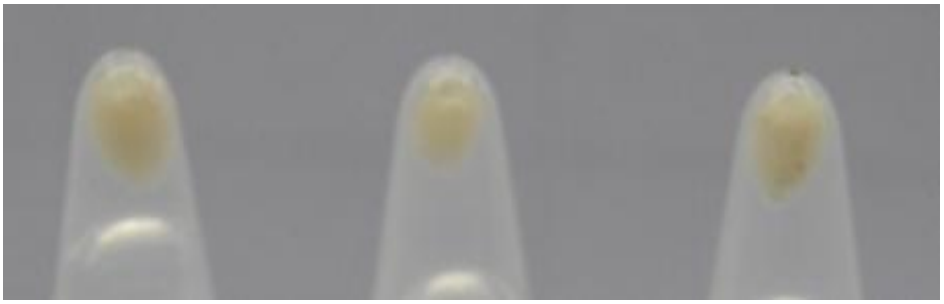


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Fig 5. Color contrast after 24 hours' cultivation (for each photo, from the left to the right are JM109, BL21 and DH5α).

A conclusion from these results was that CrtEBI has expression leakage and the constitutive promoter can enhance the expression while the terminator can prevent the leakage for some extent. From our viewpoint, 2 factors are responsible for the significant color leakage together.

First of all, transcription factor-DNA interaction may result in this phenomenon. From the energy landscape for the TF Cro on the bacteriophage λDNA (Figure 6), it is obvious that many sites on the genome can have a strong interaction with TF. Maybe there are several such sites at the start of CrtEBI and CrtEBIY, or their upstream non-coding sequence.

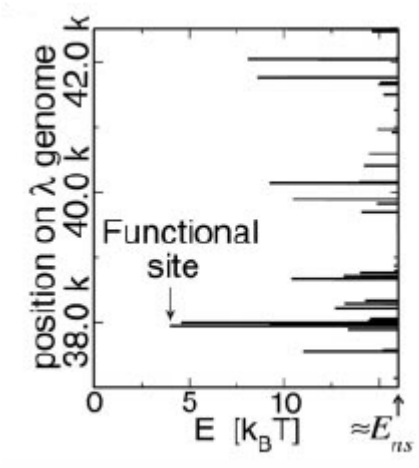


Figure 6 Energy landscape for the TF Cro on the bacteriophage λDNA(Gerland et al., 2002). The landscape appears to be random, with different strength.

Secondly, the lycopene biosynthesis is an enzymatic reaction. Lycopene biosynthesis needs

three enzymes which are GGPP synthase (produced by CrtE), phytoene synthase (produced by CrtB) and phytoene desaturase (produced by CrtI). All the Km values of these enzymes are showed in Table 1 (Takaya et al., 2003; Neudert et al., 1998; Raisig et al., 1996). From the Km value, it is obvious that a small amount leakage of enzyme will lead to a large amount of product.

Table 1 Km value for GGPP synthase, phytoene synthase, phytoene desaturase

	Km(μM)
GGPP synthase	6.78±0.99
phytoene synthase	33.3
phytoene desaturase	166

2. CrtEBIY Characterization

Biobrick K274200 is the β-carotene-producing device submitted by team Cambridge 2009. It is used to produceβ-carotene which is a yellow pigment which can be detected by the naked eye.

However, 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 7.

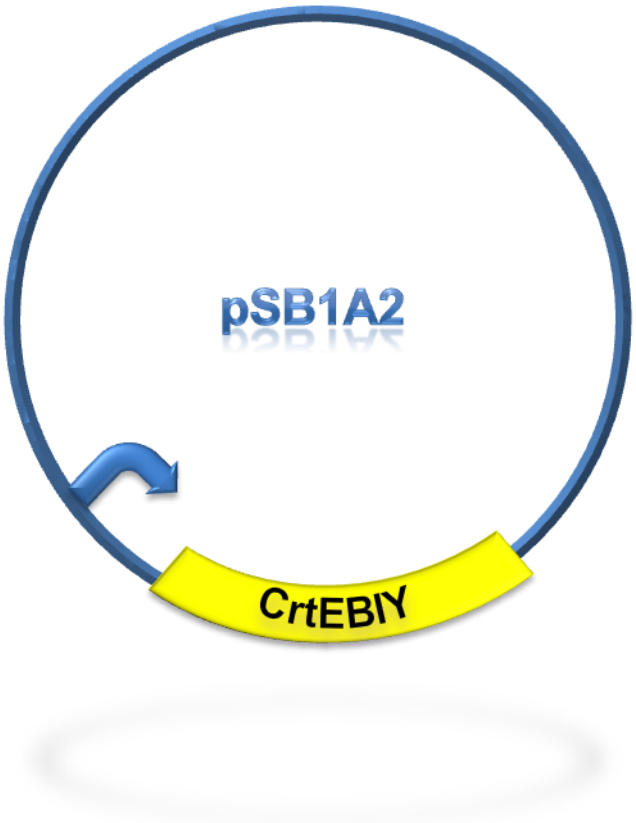
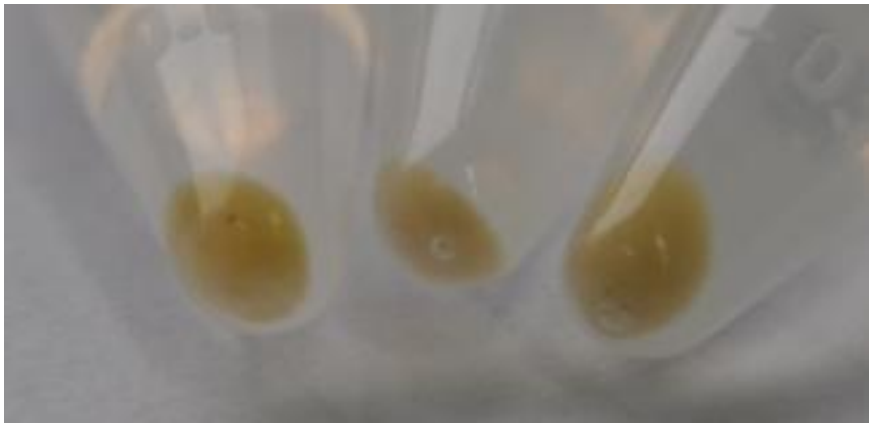


Figure 7. The biobrick we constructed to characterize biobrick BBa_K274100. It is Constitutive promoter BBa_J23103 driving CrtEBI.

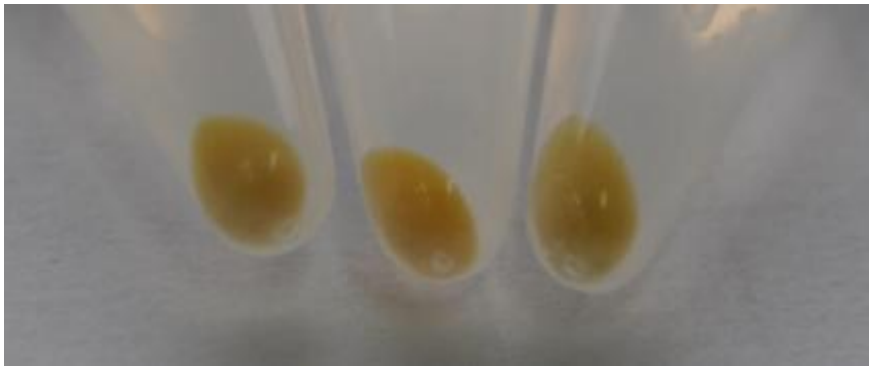
After the biobrick was constructed, it was transformed to JM109, BL21 (DE3) and DH5α, respectively. Then we compared their color with the corresponding strains that bear plasmid with

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

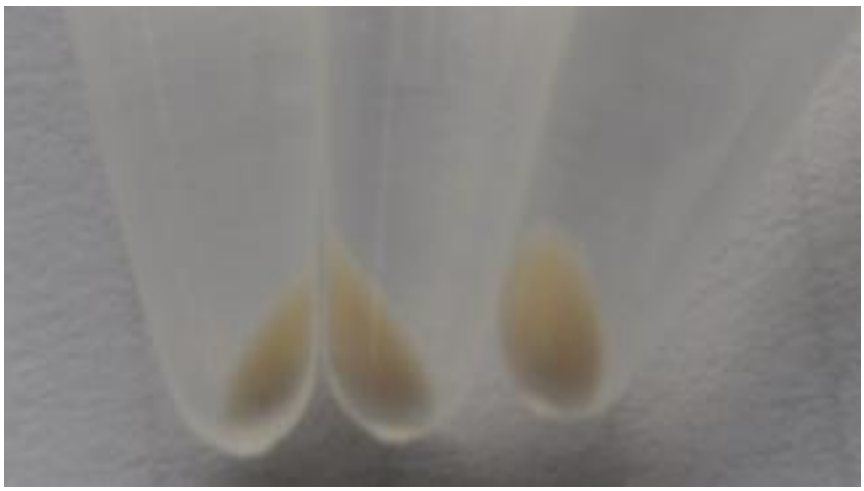


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Figure 9 **Color contrast after 12 hours' cultivation (for each photo, from the left to the right are JM109, DH5α and BL21)**.

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.

Reference

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