Best Part Collection

Overview

Throughout the progression of this project, we have utilized a diverse array of parts. Our parts include regulatory elements LacO and the regulatory gene LacI derived from the lactose operon; the gene for integrase Bxb1 gp35 capable of achieving gene inversion effects, along with its target sites attP and attB sequences; additionally, we have the gene for Bps enzyme responsible for producing chromogenic substances, and the gene for its auxiliary enzyme PPTase. These parts were all extracted anew from unprocessed genomes in NCBI, then refined and designed to create original components. Each of these parts can be replaced or used independently.

These interchangeable parts, through effective collaboration, enable us to achieve the expected outcomes for each of our modules. Among these, the several parts used to implement the visualization module demonstrate excellent cooperation and strong innovation, and can be designated as a Best Part Collection.

Three collections have emerged from our total collection, presenting a progressive relationship from simple to complex, with each capable of achieving unique effects and serving as standalone templates.

1. Bidirectional Switch Collection

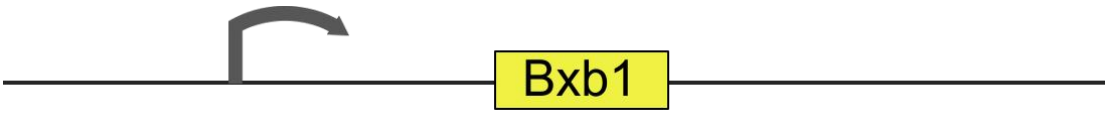


Figure 1

Figure 2

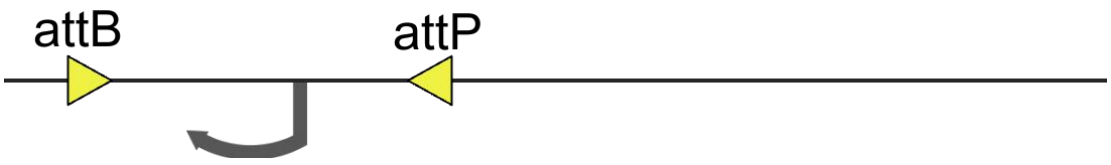


Figure 3

It consists of a regulatory operon expressing Bxb1 integrase and an effector operon containing Bxb1 enzyme target sites attP and attB. The latter has RBS sequences at both ends, so the promoter in the middle can mediate gene expression on either side regardless of its orientation.

Thus, by regulating the promoter of the first operon (Figure 1), we can switch the second operon between the two states shown in Figure 2 and Figure 3, expressing different genes respectively, thereby achieving a bidirectional switching effect.

2. Four-Segment Output Collection

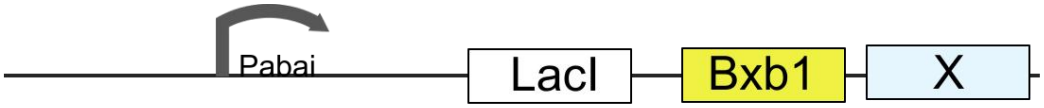


Figure 4

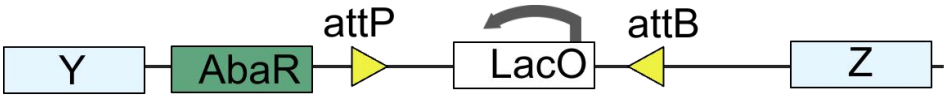


Figure 5

Figure 6

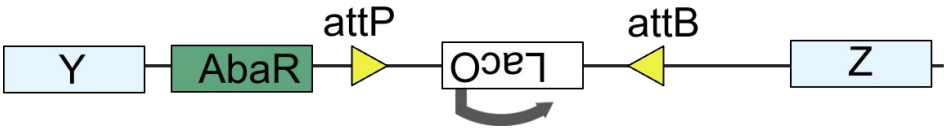


Figure 7

Building upon the previous collection, the promoter of the regulatory operon was replaced with promoter P(abaI), which can be activated by AbaR protein bound with AHL. Downstream of this promoter, the regulatory gene LacI and gene X were added. Meanwhile, the control element LacO was inserted at the promoter region of the effector operon, with the AbaR gene and gene Y added to the left side, and gene Z to the right side.

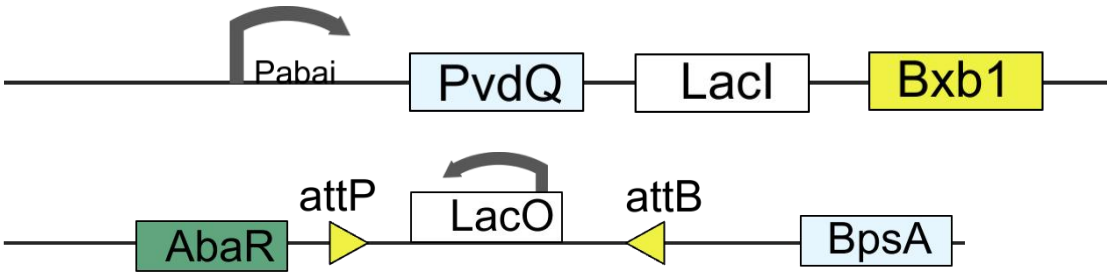
When this collection first begins operation, the effector operon expresses AbaR

and Y. Once the environmental AHL concentration exceeds the threshold, AbaR binds with AHL to activate the regulatory operon, resulting in the expression of LacI, Bxb1, and X. Subsequently, Bxb1 acts on attP and attB to invert the promoter of the effector operon, while LacI binds to LacO to repress the promoter. At this point, Y, AbaR, and Z are no longer expressed, and the regulatory operon is simultaneously shut down. When AHL concentration decreases due to various factors and falls below the threshold detectable by the regulatory operon's promoter, the expression of LacI, Bxb1, and X ceases. As LacI is gradually metabolized naturally, the effector operon reactivates and expresses Z. Through this process, the functionally active genes X, Y, and Z achieve four-segment expression:

	X	Y	Z	Period
1	−	+	−	Collection initiates operation - AHL concentration reaches threshold
2	+	+	−	- Bxb1 begins to function
3	−	−	−	- LacI gradually degrades
4	−	−	+	- Collection ceases operation

These four distinct outputs, through the sophisticated arrangement and design of genes X, Y, and Z, can be combined to construct complex logic gates, achieving diverse effects and forming more intricate biological circuits. The irreversible action of Bxb1 can also enable biological circuits to produce effects similar to one-time memory storage.

3. Visualization Presentation Collection



Building upon the previous collection, in our project we designated X as the gene for PvdQ—an enzyme capable of degrading AHL—omitted Y, and designated Z as the gene BpsA responsible for chromogenic production. Utilizing principles similar to the previous collection, we achieved segmented output: when the collection first begins operation, the effector operon expresses AbaR protein; when AbaR encounters AHL at concentrations exceeding the threshold, it binds to P(abaI), activating the regulatory operon to produce PvdQ, LacI, and Bxb1. After Bxb1 exerts its function, the effector operon inverts, and its promoter is bound by LacI. Once LacI degrades,

the promoter becomes functional and expresses BpsA. Notably, immediately following the inversion of the effector operon, sufficient quantities of Bxb1, LacI, and PvdQ have already been expressed. At this point, AbaR is no longer being produced, which conveniently allows the regulatory operon to gradually lose function as AbaR is depleted—avoiding wasteful energy expenditure that would compromise engineered bacterial viability. The cessation of AbaR production, which itself binds AHL, also prevents false positives when we subsequently assess whether AHL concentration has dropped to a certain level, as residual AbaR would otherwise bind AHL and cause misjudgment. Exquisitely elegant.

Through this mechanism, our engineered bacteria can achieve pseudo-memory: upon encountering a lesion site, they perceive sufficiently high AHL concentrations before initiating subsequent programs, and subsequently produce a blue product to report externally once AHL concentration drops to a designated level.

Basic part		
ID	**TITLE**	**FUNCTION**
BBa_J23117	weak constitutive promoter1	Initiate downstream gene transcription at a low basal level without inducers, ensure basic gene expression without heavy metabolic burden, provide transcription initiation signals for regulatory/functional proteins requiring basal expression
New part	AbaR	Belongs to the LuxR family, specifically binds AHL quorum sensing signals produced by CRAB; undergoes conformational change to form active dimers, thereby activating the transcription of pAbaI promoter, serving as the core sensor of the quorum sensing pathway
New part	pAbaI	A weak promoter by itself, only activated when bound to AHL-associated active AbaR protein to initiate downstream gene transcription, a key element linking signal perception and gene

		expression in the quorum sensing pathway
New part	PvdQ	Specifically degrades quorum sensing signal molecule AHL, blocks the quorum sensing pathway of pathogenic bacteria
New part	CL(lacI)	Mediates the binding and repression of lac promoter by LacI
New part	Bxb1(gp35)	Bxb1 phage integrase, recognizes and mediates site-specific recombination of Bxb1 attP and Bxb1 attB sites, realizes DNA fragment integration between plasmid and genome, or between plasmids
New part	Bxb1 attB	Bxb1 phage attachment site B, a paired site-specific recombination site with Bxb1 attP; undergoes irreversible site-specific recombination with attP site under the action of Bxb1 (gp35) integrase to achieve site-directed integration of DNA fragments
New part	Bxb1 attP	Bxb1 phage attachment site P, a paired site-specific recombination site with Bxb1 attB; undergoes irreversible site-specific recombination with attB site under the action of Bxb1 (gp35) integrase to achieve site-directed integration of DNA fragments
New part	P(anderson)	Anderson constitutive promoter, a standardized constitutive promoter that continuously drives downstream gene transcription with adjustable expression intensity, suitable for stably expressing functional proteins (e.g., enzymes,

		accessory proteins
New part	BpsA	Biopolymer synthase, the core catalytic enzyme; catalyzes the dimerization of glutamine to form a bright blue dipeptide pigment in one step after activation by PPTase, serving as an important effector enzyme for visualization
New part	PPTase	Phosphopantetheinyl transferase, a key accessory enzyme in the biosynthetic pathway; transfers the phosphopantetheinyl group to the active site of synthases (e.g., BpsA) to activate their catalytic function and ensure normal synthesis of biopolymers

(For a more detailed parts list for other sections, please visit our Parts section.)