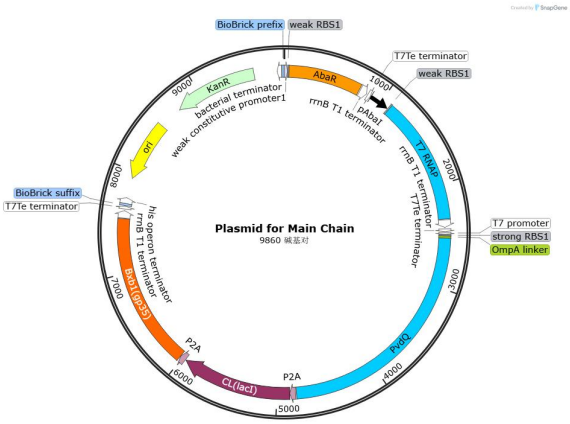
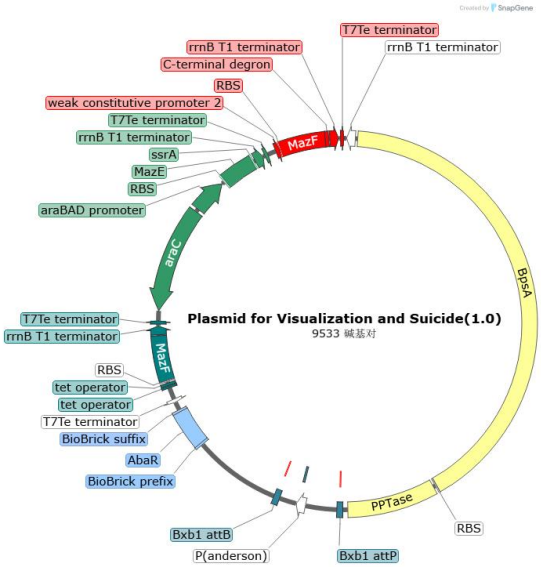
Plasmid Map：

Plasmid for main chain：



Plasmid for visualization and suicide：



Main chain:

Basic:

BBa_J23117：weak constitutive promoter1

New part：AbaR

BBa_B0015：rrnB T1 and T7Te terminator
New part：pAbaI
BBa_I716001：T7 RNAP
BBa_B0034：Weak RBS1
BBa_J64997：T7 promoter
BBa_K103007：OmpA linker
BBa_B0030：strong RBS1
New part：PvdQ

Composite:

New part：Weak constitutive promoter1→AbaR
New part：pAbaI→T7 RNAP
New part：T7 promoter→OmpAlinker→PvdQ→P2A→CL(lacI)→P2A→Bxb1(gp35)

Visualization:

Basic:

New part：CL(lacI)
New part：Bxb1(gp35)
New part：PPTase
New part：BpsA
BBa_B0030：strong RBS1
New part：P(anderson)
New part：Bxb1 attB
New part：Bxb1 attP
New part：Bxb1(gp35)
Composite:
New part：Bxb1 attB→P (anderson)→Bxb1 attP→PPTase→RBS→BpsA

Suicide:

Basic:

BBa_I0500：P_BAD(include araC)
BBa_K4060000：MazE
BBa_J23114：weak constitutive promoter 2
BBa_K4060001：MazF

BBa_R0040: P_Tet-on

New part: ssrA

New part: C-terminal degron

BBa_B0015: rrnB T1 and T7Te terminator

BBa_B0034: Weak RBS1

Composite:

New part: P_BAD→MazE→ssrA tag

New part: Weak Constitutive Promoter 2→MazF→C-terminal degron

New part: Tet-on→MazF

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
BBa_B0015	rrnB T1 and T7Te terminator	Fused with rrnB T1 and T7Te terminators, efficiently terminates RNA polymerase transcription, prevents transcriptional read-through to downstream elements, and avoids interference between different transcription units
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
BBa_I716001	T7 RNAP	Highly specific, only recognizes T7 promoter; drives high-level transcription of downstream genes upon binding; forms the T7 expression system with T7 promoter to achieve efficient and specific

		expression of target genes, serving as the core element for gene expression amplification
BBa_B0034	Weak RBS1	Located upstream of mRNA, the ribosome binding site that determines translation initiation efficiency; weak RBS reduces translation efficiency and leads to low-level expression of downstream genes
BBa_J64997	T7 promoter	Only specifically recognized and bound by T7 RNA polymerase, almost no basal expression without T7 RNAP; enables efficient transcription of downstream genes upon binding
BBa_K103007	OmpA linker	Fused to the N-terminus of target proteins, guides target proteins to localize to the E. coli outer membrane, or realizes protein surface display/secretion expression
BBa_B0030	strong RBS1	High ribosome binding efficiency, significantly improves translation initiation efficiency, and enables high-level expression of downstream genes
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	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
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
BBa_I0500	P_BAD(include araC)	AraC protein encoded by araC acts as a regulator: represses P_BAD transcription without arabinose; undergoes conformational change to activate P_BAD and drive efficient downstream gene transcription with arabinose, a precisely regulatable inducible promoter
BBa_K4060000	MazE	Antitoxin protein of the MazEF TA system; specifically binds to MazF toxin protein to form an inactive complex and neutralize MazF toxicity; an inherently unstable protein easily degraded by proteases, the core antitoxin element of the suicide switch system
BBa_J23114	weak constitutive promoter 2	Weak constitutive promoter 2, similar in function to J23117; continuously initiates low-level downstream transcription and provides a transcription initiation signal for the basal expression of MazF in the suicide switch
BBa_K4060001	MazF	Toxin protein of the MazEF TA system, a specific mRNA endonuclease that cleaves intracellular mRNA, blocks protein synthesis, and causes cell growth arrest or even death; exerts toxicity only when MazE antitoxin is absent, the core lethal element of the suicide switch system
BBa_R0040	P_Tet-on	Tetracycline-inducible promoter (Tet-on system), regulated by TetR repressor protein: represses transcription without tetracycline/aTc; TetR undergoes conformational change to relieve repression and initiate downstream gene transcription with aTc, an artificially regulatable inducible promoter serving as the "emergency switch" of the suicide switch
New part	ssrA	ssrA degradation tag, a C-terminal short peptide

		tag specifically recognized by E. coli cytoplasmic ClpXP protease, which rapidly degrades the fused protein and significantly shortens the protein half-life
New part	C-terminal degron	C-terminal degradation tag, a universal protein degradation tag slowly recognized and degraded by proteases, which slowly degrades the fused MazF toxin protein

Composite part

ID	TITLE	FUNCTION
New part	Weak constitutive promoter1 → AbaR	AbaR basal expression unit; drives continuous low-level expression of AbaR via weak constitutive promoter 1, ensures a sufficient amount of AbaR protein is always present in cells to rapidly sense environmental AHL signals, and provides a basis for signal recognition in the quorum sensing pathway
New part	pAbaI → T7 RNAP	AHL-inducible T7 RNAP expression unit, the core amplification module of the quorum sensing pathway; AbaR binds AHL to activate pAbaI promoter and drive T7 RNAP expression; the synthesized T7 RNAP then recognizes T7 promoter to achieve efficient downstream gene transcription, completing the cascade amplification of quorum sensing signal to high-level gene expression and improving the sensitivity and efficiency of signal response
New part	T7 promoter → OmpA linker → PvdQ → P2A → CL(lacI) → P2A → Bxb1(gp35)	T7-driven polycistronic co-expression unit; achieves efficient co-expression of three proteins via P2A self-cleaving peptide under the drive of T7 promoter, an integrated functional module of quorum sensing block, gene regulation and DNA integration: 1. OmpA-PvdQ: PvdQ is secreted/ localized to the outer membrane to degrade AHL and block the quorum sensing pathway of pathogenic bacteria; 2. CL(lacI): represses P(anderson), an important element for quorum

		sensing; 3. Bxb1(gp35): provides site-specific recombination function to realize DNA site-directed integration
New part	Bxb1 attB → P(anderson) →Bxb1 attP→ PPTase→RBS→BpsA	Integrates site-directed integration and biosynthetic functions to achieve stable synthesis of biopolymers: 1. Bxb1 attB/attP: undergoes site-specific recombination under the action of Bxb1(gp35) and drives P(anderson) expression after inversion; 2. P(anderson): continuously drives the expression of PPTase and BpsA after inversion; 3. PPTase+RBS+BpsA: PPTase activates the catalytic function of BpsA, and BpsA catalyzes the dimerization of glutamine to form a bright blue dipeptide pigment to complete visualization
New part	P_BAD → MazE → ssrA tag	P_BAD initiates MazE expression only in the presence of arabinose; the ssrA tag causes rapid degradation of MazE, forming a synthesis-degradation dynamic balance to ensure that MazE neutralizes MazF sufficiently; when arabinose is exhausted or the plasmid is lost, MazE synthesis stops and degrades rapidly, leading to the loss of antitoxin function and triggering cell death
New part	Weak Constitutive Promoter 2→ MazF→ C-terminal degron	The core basal toxin module of the suicide switch; weak constitutive promoter 2 drives continuous low-level expression of MazF, and the C-terminal degradation tag causes slow degradation of MazF; when MazE is absent, intracellular MazF accumulates rapidly and exerts mRNA cleavage function to trigger cell death, ensuring the response sensitivity of the suicide switch
New part	Tet-on → MazF	The artificial emergency lethal module of the suicide switch; almost no MazF expression in this pathway without tetracycline; when artificial cell death is required, aTc is added to activate the Tet-on promoter and drive high-level MazF expression, which breaks through the antitoxin threshold and kills cells rapidly even in the presence of MazE, suitable for scenarios requiring emergency termination of engineered bacteria activity