Supporting Information

A tunable genetic switch for tight control of *tac* promoters in *Escherichia coli* boosts expression of synthetic injectisomes

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Supporting experimental procedures

Plasmid construction. The plasmid pGE∆*lacI* containing the genomic homology regions flanking the genes *lacZI* was used for deleting these genes in SIEC generating the control strain SIEC∆*lacI*. To integrate the gfp^{TCD} gene (Corcoran, et al., 2010) into the *ypjA* locus of the SIEC derived strains, the plasmid pGE*ypjAPtac*GFP that contains homology regions (HRs) flanking *ypjA* locus was used. The series of plasmids pGE*csg*3R containing HRs flanking the *curli* locus *csg* were used to integrate the different versions of the regulatory circuit into this locus. The regulatory circuits were built in a modular way, so each part was flanked by an unique restriction site pair: XhoI/SacI for curli 5' homology region, SacI/BamHI for terminator T1, BamHI/PstI for cI+ ind-, PstI/SalI for *Ptet-tetR*, SalI/SacII for terminator T0, SacII/EcoRI for P_R, EcoRI/KpnI for $LacI^{W220F}$, KpnI/SpeI for 3' region of LacI and SpeI/SphI for curli 3' homology region. The original circuit (3R-I) was obtained by gene synthesis (GeneArt, ThermoFisher Scientific). When one part needed to be replaced to generate a new version of the circuit, the new part was amplified by PCR using oligonucleotides that incorporated the flanking restriction sites (Table S1), the plasmid backbone and the generated amplicon were digested with the corresponding restriction enzymes and ligated. RBS, ssrA degradation tags and *mf*-Lon tag were incorporated in the oligos as overhangs. Specifically, the *ssrA* degradation tags were incorporated as follows: the vector backbone pGE*csg*3R-I was digested using the restriction enzymes EcoRI-KpnI (to remove $lacI^{W220F}$ gene) and $lacI^{W220F}$ gene was amplified by PCR using primers incorporating the *ssrA* tag sequence into the 3' terminal region of $lacI^{W220F}$. This PCR product was then digested using the restriction enzymes EcoRI-KpnI and cloned into the previously digested vector backbone. For building pGE*csg*3R-XLon, the vector backbone of pGE*csg*3R-I was digested using the restriction enzyme BamHI and 5'-dephosphorylated using the calf-intestinal alkaline phosphatase (CIP, NEB) for further cloning the PCR product from the amplification of the *mf-Lon* gene from plasmid pECL275 (Cameron and Collins, 2014).

This PCR product (*mf-Lon* gene) was digested using the restriction enzyme BamHI (NEB) and 5'-dephosphorylated using the Calf-intestinal alkaline phosphatase (CIP, NEB). It was then cloned into the previously digested vector backbone resulting in the definitive plasmid pGE*csg*3R-XLon.

Supporting Tables.

Table S1. Oligonucleotides used in this work.

Name	Sequence (5'-3')	Use
3' del LacI confirm	ATTGATGGTGAACATGATGCCGAC	Anneals with 3' region of LacI, for deletion checking
5'_RegCircuit_integ	CGTTTGGCTGAGGATCCATTTGTC	Anneals with the 5' region of all 3R circuits, for integration checking
F_LacI_seq	GCGTCTGCGTCTGGCTGGC	For LacI sequencing
R_3' de LacI	CTAATGAGTGAGCTAACTCACATTAATTGCG	Anneals with the 3' region of lacI
F_EcoRI_RBS0034_LacI	GTTGCGAATTCAAAGAGGAGAAATACTAGATGAAACCAGTAACG	For inserting RBS0034 upstream LacI (3R-X2)
F_EcoRI_RBS0034_(GTG)LacI	GTTGCGAATTCAAAGAGGAGAAATACTAGGTGAAACCAGTAACG	For inserting RBS0034 upstream LacI and changing its start codon to GTG (3R-X3)
R_KpnI_LacI	TGCGCGGTACCTCACTGCCCGCTTTCCAGTCG	For amplifying LacI without tags
R_KpnI_ssrAtag(LAA)_LacI	TGCGCGGTACCTCAAGCAGCCAGAGCGTAGTTTTCGTCGTTAGCAGCCTGCCCGCTTTCCAGTCGG	For fusing the ssrAtag(LAA) to LacI 5' end (3R-L)
R_KpnI_ssrAtag(ASV)_LacI	TGCGCGGTACCTCAAACTGATGCAGCGTAGTTTTCGTCGTTTGCTGCCTGCCCGCTTTCCAGTCGG	For fusing the ssrAtag(ASV) to LacI 5' end (3R-X)
R_KpnI_ssrAtag(AAV)_LacI	TGCGCGGTACCTCAAACTGCTGCAGCGTAGTTTTCGTCGTTTGCTGCCTGCCCGCTTTCCAGTCGG	For fusing the ssrAtag(AAV) to LacI 5' end (3R-A)
ypjA_5'	AGCTGTGCGAACGTGGTATTAACTTTC	Anneals with the 5' region of ypjA, for integration checking
ypjA_3'	AACAACACTATGGCCTGACACTGAACG	Anneals with the 5' region of ypjA, for integration checking
F_mf-lon_BamHI	TGGCTGAGGATCCATTAAAGAGGAGAAATACTAGATGAGTAAAAAAATCAAACTACCTATTTTCC	For cloning mf-lon protease in 3R-XLon
R_mf-lon_BamHI	ACAAATGGATCCTTATTTTGTTTTAAAAACTATTGCAAATACCTC	For cloning mf-lon protease in 3R-XLon
F_seq_mflon_1678	GTCACATCAACTCTATCATCCGTAAA	For sequencing mf-lon protease in 3R-XLon

Table S2. Antibodies used in this work.

Antigen	Primary antibody	Secondary Antibody
LacI	anti-LacI mAb (1:1000). Millipore	Anti-mouse IgGs-peroxidase (1:5000). Sigma
EscC	Rabbit polyclonal anti EscC EPEC (1:1000). (Ruano-Gallego, et al., 2015)	Protein A-peroxidase (1:5000). ThermoFisher
EspA	Rabbit polyclonal anti EspA EPEC (1:2000). Gad Frankel Lab.	Protein A-peroxidase (1:5000). ThermoFisher
EspB	Rabbit polyclonal anti EspB EPEC (1:2000). González-Pedrajo Lab.	Protein A-peroxidase (1:5000). ThermoFisher
GroEL	anti-GroEL mAb-peroxidase (1:5000). Sigma	--

Supporting References

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