Title: The Bacteriophage Lambda CII Phenotypes for Complementation, Cellular Toxicity, and Replication Inhibition Are Suppressed in *cII-oop* Constructs Expressing the Small RNA OOP.

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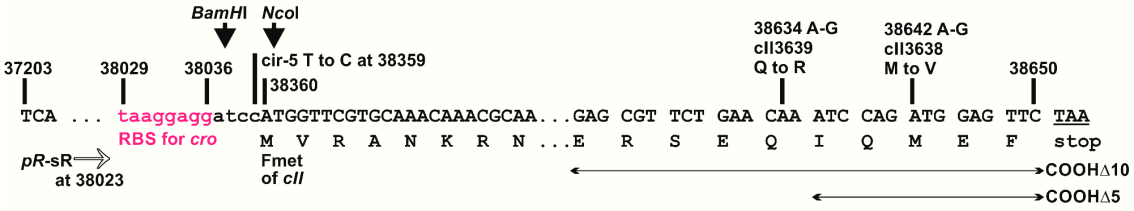
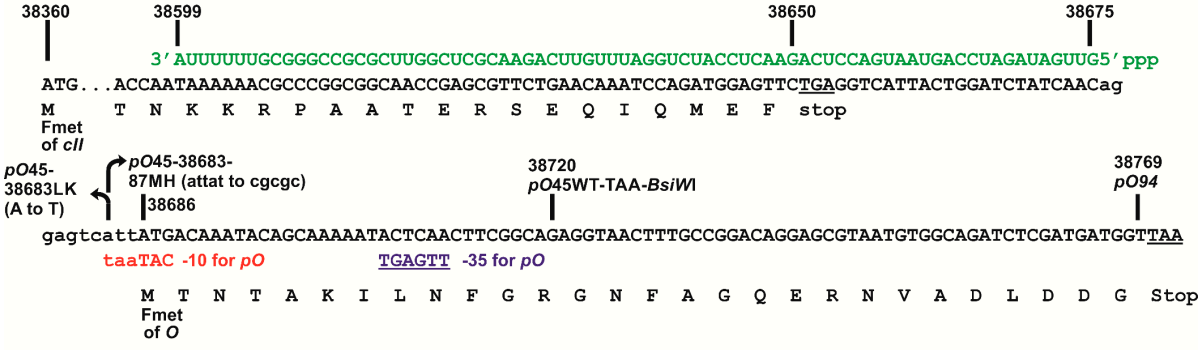


Plasmids 2: cII-oop-pO94, cII-oop-pO45, cII-oop-pO-38383LK, cII-oop-pO45-38683-87MH



Plasmids 3: cII-cy3048, cII-cy2001, cII-cy42, cII-cy3001, sR-38339pE-cII, sR-38339pE-cII-oop-pO94

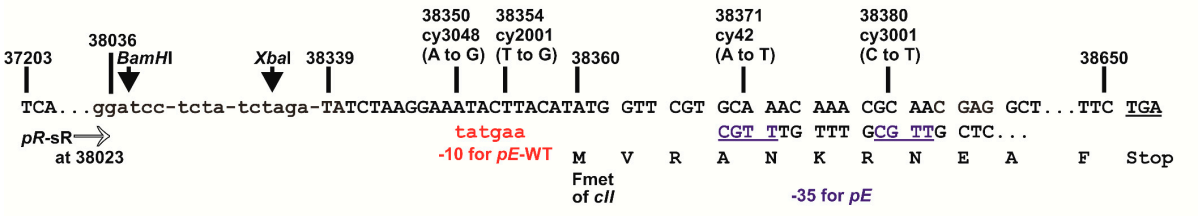


Figure S1. Sequence variations (1-3) of pcIpR-cII-timm enabling *cII* expression arrangements. The point mutations incorporated within *cII* or promoter *pO* were described in [1-4]. The sequences in *magenta* are RBS. For the plasmids in sequences 1 & 2 the RBS is identical to 8 bases of the RBS consensus sequence for gene *cro*. Sequences in *red* are "-10" promoter regions (for *pO*, in plasmid sequence 2; for *pE*, in plasmid sequence 3). The sequences in *blue* (underlined) are for "-35" region of *pO* promoter (sequence 2) and the suggested -35 region for the *pE* promoter (in sequence 3) within the N-terminal sequence of *cII*. The sequence in *green* (shown sequence 2) represents the *oop* sequence encoding OOP RNA. The Plasmids 1 include: cII, cII_{1-92} (deleting five amino acids from the carboxy-terminal end of *cII*), cII_{1-}87, cII_{1-77}, cII_{1-67}, cII_{1-57}, cII_{1-47}, cII-3638, cII-3639, cII-3638+3639. The sequence for Plasmids 2 include the sequence 1 for WT *cII* plus the *oop* sequence that overlaps the carboxyl end of *cII*, plus the *pO* sequence that overlaps with the sequence of N-terminal end of gene *O*: cII-oop-pO94, cII-oop-pO45, cII-oop-pO45-38383LK, cII-oop-pO-38683-87MH. The sequence for Plasmids 3 include the WT *pE* promoter from sR-38339-pE-cII, sR-38339pE-cII-oop-pO94 and *pE* mutations cII-cy3048, cII-cy2001, cII-cy42, and cII-cy3001.

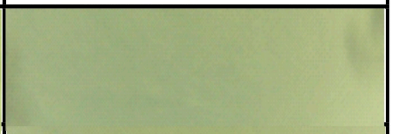
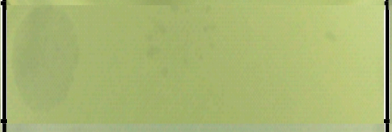
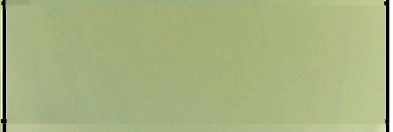
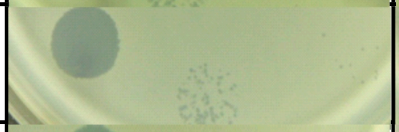
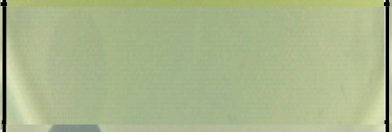
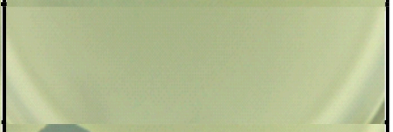
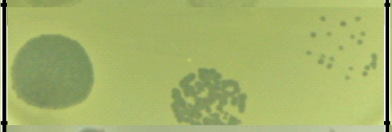
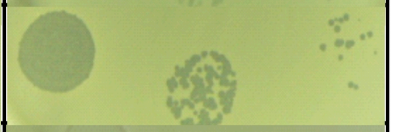
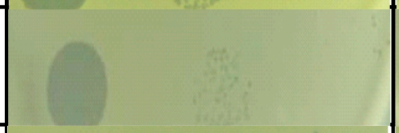
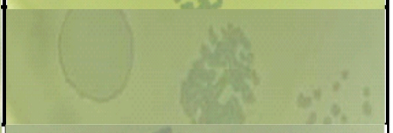
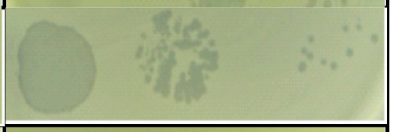
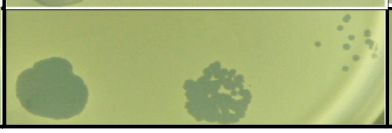
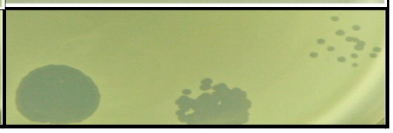
Plasmid in host cells	λ*imm*434*cII*2002 pfu spotted per overlay plate incubated at 30°C			37°C			39°C		
	~16,000	~160	~16	~16,000	~160	~16	~16,000	~160	~16
594 [*cII*]									
594 [cII_{1-92}]									
594 [cII_{1-87}]									
594 [cII_{1-77}]									
594 *hflA*::kan [cII]									
594 *pcnB*::kan[cII]									
594 *rpoB* B8 [cII]									
594 *rpoB* D2 [cII]									
594 *rpoB* D2 [cII_{1-87}]									

Figure S2. Deletions in *cII,* or host mutations influencing CII complementation *in trans*. Complementation of the *cII*-defect on the phage by the *cII* allele expressed from the plasmid was assessed by the ability of the spotted phage to form clear, turbid, or no pfu at 37 and 39°C. High expression of the CI_{434} repressor made from the phage will fully repress its *o*L (not assayed) and *o*R transcription, preventing phage growth (pfu formation). An intermediate level of *cII* expression from the plasmid at 37°C can permit the otherwise clear plaque forming λ*imm*434 *cII*68 and λ*imm*434*cII*2002 phages (seen at 30°C) to form turbid plaques at 37°C. In contrast, the expression of the *cII* allele on the plasmid is blocked when the Ts CI857 repressor is active in cells grown at 30°C.

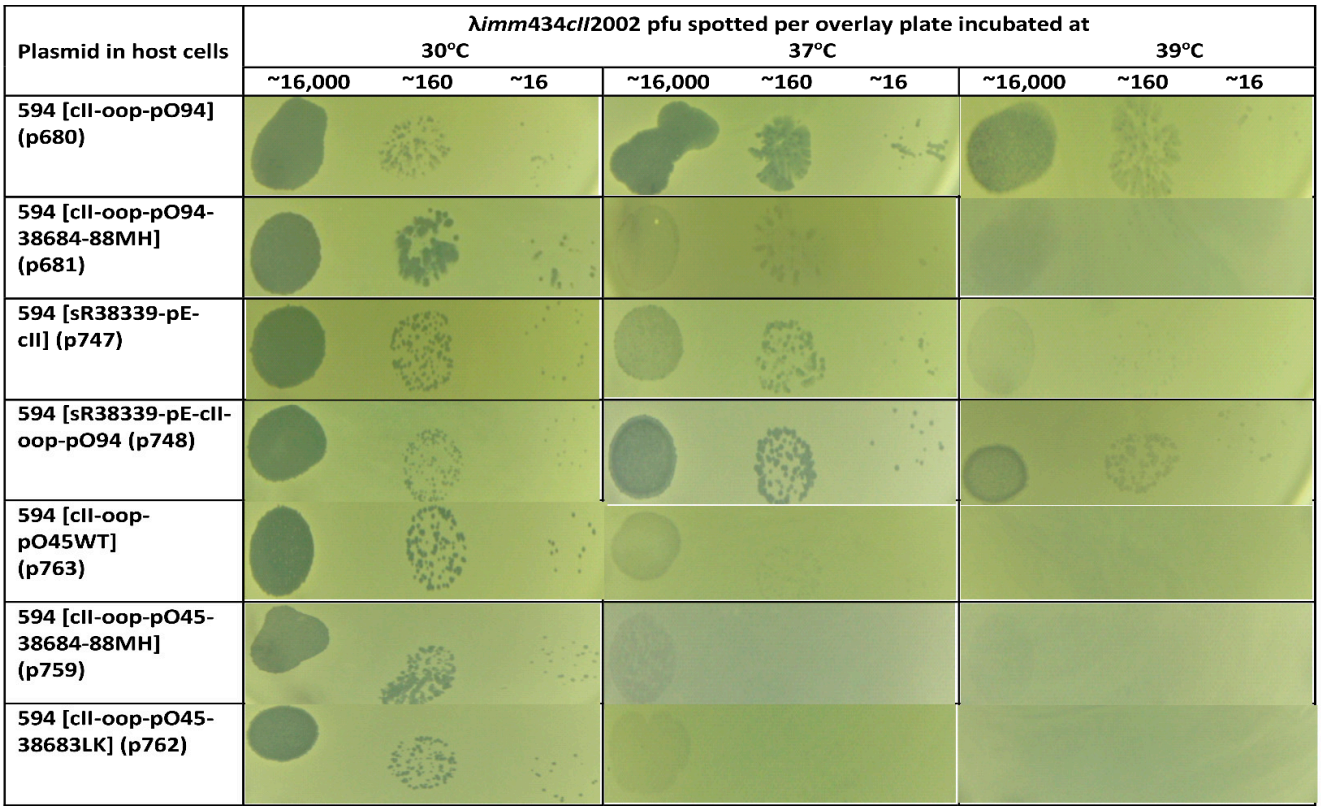


Figure S3. Influence of *pO* variations on CII complementation *in trans*.

Supplemental tables

Table S1. Gene expression units cloned within plasmid vector pcIpR-[]-timm.

Plasmid	Strain	Construction primer/ method employed
cII	**p679**	**L-Bam-cII and R-ClaBsi-cII copy from p27R**
cII-oop-pO94	**p680**	**L-Bam-cII and R-Bsi-O-Po copy from p27R**
cII-oop-pO94-38683-87MH	**p681**	**L-Bam-cII and R-Bsi-O-Po copy from p27RpO$^-$**
cII$_{1-92}$	**p696**	**L-Bam-cII and R-Cla-Bsi-CIIΔ15**
cII$_{1-87}$	**p732**	**L-Bam-cII and R-Cla-Bsi-CIIΔ30**
cII$_{1-77}$	**p715**	**L-Bam-cII and R-Cla-Bsi-CIIΔ60**
cII$_{1-67}$	**p721**	**L-Bam-cII and R-Cla-Bsi-CIIΔ90**
cII$_{1-57}$	**p723**	**L-Bam-cII and R-Cla-Bsi-CIIΔ120**
cII$_{1-47}$	**p724**	**L-Bam-cII and R-Cla-Bsi-CIIΔ150**
cII-oop-pO45WT	**p763**	**L-Bam-cII and R-Bsi45poWT**
cII-oop-pO45-38383LK	**p762**	**L-Bam-cII and R-Bsi45po38383LK**
cII-oop-pO45-38683-87MH	**p759**	**L-Bam-cII and R-Bsi45po38684-88MH**
cII-3638	**p756**	**L-Bam-cII and R-Bsi-cII-3638**
cII-3639	**p757**	**L-Bam-cII and R-Bsi-cII-3639**
cII-3638+3639	**p758**	**L-Bam-cII and R-Bsi-3638+3639**
sR-38339-cII-cy3048	**p756**	**LXbaI-38339-cy3048 and R-ClaBsi-cII**
sR-38339-cII-cy2001	**p765**	**LXbaI-38339-cy2001 and R-ClaBsi-cII**
sR-38339-cII-cy42	**p764**	**LXbaI-38339-cy42 and R-ClaBsi-cII**
sR-38339-cII-cy3001	**p766**	**LXbaI-38339-cy3001 and R-ClaBsi-cII**
sR-38339-pE-CII	**p747**	**LBamXba38339 and R-nopo-Bsi-Cla (with WT cII stop)**
sR-38339-pE-cII-oop-pO94	**p748**	**LBamXba38339 and R-Bsi-O-po (with WT cII stop)**

Table S2. Oligonucleotides used for plasmid construction and sequencing.

Primer Name	5' to 3' Sequence
L37904+18	**gctgctcttgtgttaatgg**
R-153-19	**gaagacagtcataagtgcgg**
L-Bam-CII	**atatggatccatggttcgtgcaaacaaacgcaacgaggctctacg**
R-ClaBsi-CII	**atatatcgatcgtacgttagaactccatctggatttgttcagaacgctcgg**
R-Bsi-45poWT	**atat cgtacgttactgccgaagttgagtatttttgctgtatttgtcataatgactcctgt**
R-Bsi-45po38383LK	**atatcgtacgttactgccgaagttgagtatttttgctgtatttgtcataaAgactcctgt**
R-Bsi-45po38684-88MH	**atatcgtacgttactgccgaagttgagtatttttgctgtatttgtcGCGCGgactcctgt**
R-Cla-Bsi-CII	**atatatcgatcgtacgttagaactccatctggatttgttcagaacgctcgg**
R-Cla-Bsi-CIIΔ15	**atatatcgatcgtacgttattgttcagaacgctcggttgccgccgggcgtttttt**
R-Cla-Bsi-CIIΔ30	**atatatcgatcgtacgttaggttgccgccgggcgtttttttattggtgagaatcgc**
R-Cla-Bsi-CIIΔ60	**atatatcgatcgtacgttaaatcgcagcaacttgtcgcgccaatcgagccatgtc**
R-Cla-Bsi-CIIΔ90	**atatatcgatcgtacgttacatgtc gtcgtcaacgacccccattcaagaacagc**
R-Cla-Bsi-CIIΔ120	**atatatcgatcgtacgttaaacagcaagcagcattgagaactttggaatccagtc**
R-Cla-Bsi-CIIΔ150	**atatatcgatcgtacgttaccagtccctcttccacctgctgatctgcgacttatc**

L-BamXba38339new	**atatggatcctctatctagatatctaaggaaatacttacatatggttcgtgc**
R-noPo-Bsi-ClaI	**atatatcgatcgtacgtcagaactccatctggatttgttcagaacgctcggt**
R-Bsi-O-Po	**atatcgtacgttaaccatcatcgagatctgccacattacgctcctgtccggc**
R-Bsi-CII-3638	**atatcgtacgttagaactccaCctggatttgttcagaacgctcgg**
R-Bsi-CII-3639	**atatcgtacgttagaactccat ctggattCgttcagaacgctcgg**
R-Bsi-3638+3639	**atatcgtacgttagaactccaCctggattCgttcagaacgctcgg**
LXbaI-38339-cy3048	**atattctagatatcttaggaaGtacttacatatggttcg**
LXbaI-38339-cy2001	**atattctagatatcttaggaaatacGtacatatggttcgtgc**
LXbaI-38339-cy42	**atattctagatatcttaggaaatacttacatatggttcgtgcTaacaaacgcaacgaggc**
LXbaI-38339-cy3001	**atattctagatatcttaggaaatacttacatatggttcgtgcaaacaaacgTaacgaggctctacgaatc**
L-Bam-OOP#1	**atatggatcctggctcgattggcgcgacaagt**
L-Bam-OOP#2	**atatggatccgttgacgacgacatggctcgat**
R-Cal-O	**atatatcgattatagatccaccccgtaaatccagtc**
R-ClaI-36P	**atatatcgattacctgctgtacctgcggcttttcgtcg**
R-ClaI-63P	**atatatcgattacttcgttctggtcacggttagcc**

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