

## SUPPLEMENTAL DATA

### Intron-assisted, viroid-based production of insecticidal circular double-stranded RNA in *Escherichia coli*

Beltrán Ortola<sup>a</sup>, Teresa Cordero<sup>a</sup>, Xu Hu<sup>b</sup> and José-Antonio Daròs<sup>a</sup>

<sup>a</sup>Instituto de Biología Molecular y Celular de Plantas (Consejo Superior de Investigaciones Científicas-Universitat Politècnica de Valencia), Valencia, Spain; <sup>b</sup>Corteva Agriscience, Johnston, Iowa, USA

**CONTACT:** José-Antonio Daròs, [jadaros@ibmcp.upv.es](mailto:jadaros@ibmcp.upv.es), IBMCP (CSIC-Universitat Politècnica de Valencia), Avenida de los Naranjos s/n, 46022 Valencia, Spain

**Supplemental Dataset S1.** Nucleotide sequences and elements of plasmids pLELVd, pLELVd-BZB, pLPP, pLELVd-DvSSJ1, pLELVdPIE-DvSSJ1, pLPIE-DvSSJ1, p15LtRnlSm, and p15CAT.

>pLELVd (2050 bp)

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CGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAAAAAATATTCTCAACATAAAAACTTTG
TGTAATACTTGTAAACGCTGCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTT
CGCCATGGGTCGGGACTTTAAATTCGGAGGATTCGTCTTTAAACGTTCCCTCCAAGAGTCCCTTCCCCAACCCCT
TACTTTGTAAGTGTGGTTTCGGCGAATGTACCGTTTCGTCTTTTCGGACTCATCAGGAAAGTACACACTTTCGGA
CGGTGGGTTTCGTTCGACACCTCTCCCCCTCCAGGTAATATCCCTTTCAAGGATGTGTTCCTTAGGAGGGTGGGT
GTACCTCTTTTGGATTGCTCCGGCCTTCCAGGAGAGATAGAGGACGACCTCTCCCATAGGGTGGTGTGTGCCAC
CCCTGATGAGACCGAAAGGTCGAAATGGGGGAAATCATCTTAGCGAAAGCTAAGGATTTTTTTTATCTGAAATG
CGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGC
GAAACCCGACAGGACTATAAAGATAACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCGACCC
TGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGT
ATCTCAGTTCCGTTGTAGGTTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCGACCGCTGCG
CCTTATCCGGTAACTATCGTCTTGGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTA
ACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTA
GAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCG
GCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTC
AAGAAGATCTTTTTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAG
TTGCCTGACTCCCCGTCTGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATAC
CGCGAGAGCCACGCTCACCAGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTG
GTCCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTTCGCCAGTTA
ATAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCA
GCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTTC
CTCCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCAGCTGCATAAATTCCTTA
CTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGATGC
GGCGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCA
TCATTGGAAAACGTTCTTCGGGGCGAAAACCTCAAGGATCTTACCCTGTTGAGATCCAGTTTCGATGTAACCCA
CTCGTGCACCCAACTGATCTTACGATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCCAAA
ATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAA
GCATTTATCAGGGTTATTGTCTCAT
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In **red**, *E. coli* murein lipoprotein promoter. In **bold**, ELVd cDNA (C327 to G46 of AJ536613), with the repeated hammerhead ribozyme domain **highlighted in yellow**, and their self-cleavage sites **underlined**. In **fuchsia**, *E. coli* ribosomal *rrnC* terminator. In **gray**, pUC replication origin. Highlighted in light grey, ampicillin resistance gene (in reverse orientation), with the promoter highlighted in dark gray).

>pLELVd-BZB (2574 bp)

CGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAAAAAATATTCTCAACATAAAAACTTTG  
TGTAATACTTGTAAACGCTG**CCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGG**TTT  
CGCCATGGGTGGGACTTTAAATTCGGGAGGATTTCGTCTTTTAAACGTTCTTCCAAGAGTCCCTTCCCCAAACCTT  
TACTTTGTAAAGTGTGGTTCGGCGAATGTACCGTTTCGTCTTTTCGGACTCATCAGGGAAAAGTACACACTTTCCGA  
CGGTGGGTTTCGTTCGACACCTCTCCCCCTCCCAGGTACTATCCCTT**GGGTCTTC**GCGGGAAAAGCGGGCAGTGAGC  
GCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGT  
TGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCGCGCAATTA  
ACCCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCCCCCTCGAGGTGACGGTATCGATAAGCTTGATATCG  
AATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGCCGCCACCGCGGTGGAGCTCCAATTCGCCCTATAGT  
GAGTCGTATTACGCGCGCTCACTGGCCGTCGTTTTACAACCGTCGTGACTGGGAAAACCTTGGCGTTACCCAATT  
AATCGCCTTGACGCACATCCCCCTTTCCGCCAGCTGGCGTAATAGCGAAGAGGCCCGACCGATCGCCCTTCCCAA  
CAGTTGCGCAGCCTGAATGGCGAATGGGACGCGGGCG**GAAGAC**GC**TCAAGGATGTGTTCCTTAGGAGGGTGGGTG**  
**TACCTCTTTTTGGATTGCTCCGGCCTTCCAGGAGAGATAGAGGACGACCTCTCCCCATAGGGTGGTGTGTGCCACC**  
**CCTGATGAGACCGAAAGGTCGAAATGGGG**GAAATCATCCTTAGCGAAAGCTAAGGATTTTTTTTTATCTGAAATGC  
GTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCG  
AAACCCGACAGGACTATAAAGATAACCAGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTT  
GCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTA  
TCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGACCGCTGCGC  
CTTATCCGTAACCTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAA  
CAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAG  
AAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGG  
CAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCA  
AGAAGATCCTTTTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGT  
TGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACC  
GCGAGAGCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGG  
TCCTGCAACTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTTCGCCAGTTAA  
TAGTTTGGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCAG  
CTCCGTTTCCCAACGATCAAGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCC  
TCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTACTGTTATGGCAGCAGCTGCATAAATCTCTTAC  
TGTCATGCCATCCGTAAGATGCTTTTTCTGTGACTGGTGTGACTCAACCAAGTCATTCTGAGAATAGTGTATGCG  
GCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGATAATACCGCGCCACATAGCAGAAGCTTTAAAAGTGCTCAT  
CATTGGAACCGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCAC  
TCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAA  
TGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTTCAATATTATTGAAG  
CATTATCAGGGTTATTGTCTCAT

In red, *E. coli* murein lipoprotein promoter. In bold, (C327 to G46 of AJ536613), with the repeated hammerhead ribozyme domain **highlighted in yellow**, and their self-cleavage sites **underlined**. In blue cDNA coding for LacZ gene inserted between T245 and T246 of ELVd cDNA. **Highlighted in dark blue**, recognition sites for type-IIS restriction enzyme BpiI, with the cleavage sites in **underlined bold**. In fuchsia, *E. coli* ribosomal rrnC terminator. In gray, pUC replication origin. **Highlighted in light grey**, ampicillin resistance gene (in reverse orientation), with the promoter **highlighted in dark gray**).

>pLPP (1916 bp)

CGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAAAAAATATTCTCAACATAAAAACTTTG  
TGTAATACTTGTAAACGCTG**GGAGACCGCGG**CAGGAAACAGCTATGACCATGATTACGCCAAGCGCGCAATTAACC  
CTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCCCCCTCGAGGTGACGGTATCGATAAGCTTGATATCGAAT  
TCCTGCAGCCCGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCAATTCGCCCTATAGTGAG  
TCGTATTACGCGCGCTCACTGGCCGTCGTTTTACAGGCG**GGTCTCG**GAAATCATCCTTAGCGAAAGCTAAGGATTT  
TTTTTTATCTGAAATGCGTTGCTGGCGTTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGC  
TCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAACCAGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCCT  
TCTCCTGTTCCGACCTGCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAT  
AGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGTTTCGTCCAAGCTGGGCTGTGTGCACGAACCCCGCTT  
CAGCCCGACCGCTGCGCTTATCCGGTAACCTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTG  
GCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCTT  
AACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTT

GGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGC  
 AGAAAAAAGGATCTCAAGAAGATCCTTTTTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTA  
TTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCC  
 AGTGCTGCAATGATACCGCGAGAGCCACGCTCACC GGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGG  
 GCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTA  
 AGTAGTTCGCCAGTTAATAGTTTGGCGAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTT  
 GGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCG  
 GTTAGCTCCTTCGGTCTCCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCA  
 CTGCATAATTCTCTTACTGTCAATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTC  
 TGAGAATAGTGTATCGCGGACCGAGTTGCTCTTCCCGCGTCAATACGGGATAATACCGCCACATAGCAGA  
 ACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCCGGGCGAAAACTCTCAAGGATCTTACCCTGTTGAGATCC  
 AGTTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGGGTGAGCA  
 AAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTT  
 TTTCATATTATTGAAGCATTATCAGGGTTATTGTCTCAT

In **red**, *E. coli* murein lipoprotein promoter. In **bold blue**, polylinker with double recognition sites for the type IIS enzyme BsaI (**highlighted in yellow**, with the cleavage sites underlined) separating the polylinker from the plasmid pBSIKS + (in reverse, in *blue italics*). In **fuchsia**, *E. coli* ribosomal *rrnC* terminator. In **gray**, pUC replication origin. Highlighted in light grey, ampicillin resistance gene (in reverse orientation), with the promoter **highlighted in dark gray**).

>pLELVd-DvSSJ1 (2649 bp)

CGATGCTTCTTTGAGCGAACGATCAAAAAATAAGTGCCTTCCCATCAAAAAATATTCTCAACATAAAAACTTTG  
 TGTAAACTTGTAAACGCTG**CCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTT**  
**CGCCATGGGTCCGGACTTTAAATTCGGAGGATTTCGTCTTTAAACGTTCTTCCAAGAGTCCCTTCCCCAAACCTT**  
**TACTTTGTAAGTGTGGTTCGGCGAATGTACCGTTTCGTCTTTCCGACTCATCAGGGAAAAGTACACACTTTCCGA**  
**CGGTGGGTTCGTGCACACCTCTCCCCCTCCAGGTAATCCCTTACCATTGTCTGAAATTTGCTGAAGTTGGT**  
**GATCAATTTGATATGTCTCATCTTGTACCGAACCGGATATCAAGGCTACTTCTTATGACTCTCTAAATAGCAATA**  
 TTTACCTTTGGAGGGAAAAGTTATCAGGCATGCACCTGGTAGCTAGTCTTTAAACCAATAGATTGCATCGGTTTA  
 AAAGGCAAGACCGTCAAATTCGGGAAAGGGGTCACAGCCGTTCCAGTACCAAGTCTCAGGGGAAACTTTGAGAT  
 GGCCTTGCAAAGGGTATGGTAATAAGCTGACGGCATGGTCTTAACCACGCAGCCAAGTCTAAGTCAACAGATC  
 TTCTGTTGATATGGATGCAGTTCACAGACTAAATGTCGGTCCGGGAAGATGATTCTTCTCATAAGATATAGTCG  
 GACCTCTCCTTAATGGGAGCTAGCGGATGAAGTGATGCAACACTGGAGCCGCTGGGAACATAATTTGTATGCGAAA  
 GTATATTGATTAGTTTTGGAGTACTCGTAAGGTAGCAAGAAGTAGCCTTGATATCCGGTTCGGTACAAGATGAGA  
 CATATCAAATTTGATCACCAACTTCAGCAATTTCAGGACAATGGT**TCAAGGATGTGTTCCCTAGGAGGGTGGGTGT**  
**ACCTCTTTTGGATTGCTCCGGCCTTCCAGGAGAGATAGAGGACGACCTCTCCCCATAGGGTGGTGTGCCACCC**  
**CTGATGAGACCGAAAGGTCGAAATGGGGGAAATCATCTTAGCGAAAGCTAAGGATTTTTTTTATCTGAAAT**GCG  
 TTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGA  
 AACCCGACAGGACTATAAAGATAACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCCTG  
 CCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTAT  
 CTCAGTTCCGGTGTAGGTGCTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCCAGCCGACCGCTGCGCC  
 TTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAC  
 AGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCTAACTACGGCTACACTAGA  
 AGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGC  
 AAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAA  
 GAAGATCCTTTTTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTTCATCCATAGTT  
 GCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCG  
 CGAGAGCCACGCTCACC GGCTCCAGATTTATCAGATAAACCAGCCAGCCGGAAGGCCGAGCGCAGAAAGTGGT  
 CCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTTGTTGCCGGAAGCTAGAGTAAGTACTGAGTTCGCCAGTTAAT  
 AGTTTGGCGAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTTGGTATGGCTTCATTCAGC  
 TCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCT  
 CCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACT  
 GTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGTGACTCAACCAAGTCATTTCTGAGAATAGTGTATGCGG  
 CGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATC  
 ATTGGAAAACGTTCTTCCGGGCGAAAACTCTCAAGGATCTTACCCTGTTGAGATCCAGTTCGATGTAACCCACT  
 CGTGCACCCAACTGATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAAT  
 GCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTTCAATATTATTGAAGC  
 ATTTATCAGGGTTATTGTCTCAT

In **red**, *E. coli* murein lipoprotein promoter. In **bold**, (C327 to G46 of AJ536613), with the repeated hammerhead ribozyme domain **highlighted in yellow**, and their self-cleavage sites **underlined**. **Highlighted in green**, inverted repeat of an 83 nt fragment of the *DvSSJ1* gene inserted between T245 and T246 of ELVd cDNA. In **green**, between the two copies of the *DvSSJ1* gene, the cDNA of the group-I *Tetrahymena termophila* 26S rRNA intron, with the 10 nt of both flanking exons **underlined**. In **fuchsia**, *E. coli* ribosomal *rrnC* terminator. In gray, pUC replication origin. **Highlighted in light grey**, ampicillin resistance gene (in reverse orientation), with the promoter **highlighted in dark gray**).

>pLELVdPIE-DvSSJ1 (3080 bp)

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CGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAAAAATATTCTCAACATAAAAACTTTG
TGTAATACTTGTAAACGCTGCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTT
CGCCATGGGTGGGACTTTAAATTCGGAGGATTTCGTCTTTAAACGTTCTCCAAGAGTCCCTTCCCCAAACCTT
TACTTTGTAAGTGTGGTTCGGCGAATGTACCGTTTCGTCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGA
CGGTGGGTTTCGTGCACACCTCTCCCCCTCCAGGTACTATCCCTTCTTCTGTTGATATGGATGCAGTTCACAGA
CTAAATGTCGGTCGGGGAAGATGTATTCTTCTCATAAGATATAGTCGGACCTCTCCTTAATGGGAGCTAGCGGAT
GAAGTGATGCAACACTGGAGCCGCTGGGAACATAATTTGTATGCGAAAGTATATTGATTAGTTTTGGAGTACTCGT
AAGGTAGCACCATTGTCCTGAAATTGCTGAAGTTGGTGATCAATTTGATATGTCATCTTGTACCGAACCGGAT
ATCAAGGCTACTTCTTATGACTCTCTAAATAGCAATATTTACCTTTGGAGGGAAAAGTTATCAGGCATGCACCTG
GTAGCTAGTCTTTAAACCAATAGATTGCATCGGTTTAAAAGGCAAGACCGTCAAATTCGGGGAAAAGGGTCAACA
GCCGTTTCAGTACCAAGTCTCAGGGGAAACTTTGAGATGGCCTTGCAAAGGGTATGGTAATAAGCTGACGGACATG
GTCCTAACCCACGCAGCCAAGTCTTAAGTCAACAGATCTTCTGTTGATATGGATGCAGTTCACAGACTAAATGTGC
GTCGGGGAAGATGTATTCTTCTCATAAGATATAGTCGGACCTCTCCTTAATGGGAGCTAGCGGATGAAGTGATGC
AACACTGGAGCCGCTGGGAACATAATTTGTATGCGAAAGTATATTGATTAGTTTTGGAGTACTCGTAAGGTAGCAA
GAAGTAGCCTTGATATCCGGTTCGGTACAAGATGAGACATATCAAATTGATCACCAACTTCAGCAATTCAGGAC
AATGGTATGACTCTCTAAATAGCAATATTTACCTTTGGAGGGAAAAGTTATCAGGCATGCACCTGGTAGCTAGTC
TTTTAAACCAATAGATTGCATCGGTTTAAAAGGCAAGACCGTCAAATTCGGGGAAAAGGGTCAACAGCCGTTTCACT
ACCAAGTCTCAGGGGAAACTTTGAGATGGCCTTGCAAAGGGTATGGTAATAAGCTGACGGACATGGTCTTAACCA
CGCAGCCAAGTCTTAAGTCAACAGATTCAAGGATGTGTTCCCTAGGAGGGTGGGTGTACCTCTTTTTGGATTGCTC
CGGCCTTCAGGAGAGATAGAGGACGACCTCTCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGT
CGAAATGGGGGAAATCATCTTAGCGAAAGCTAAGGATTTTTTTTATCTGAAATGCGTTGCTGGCGTTTTTCCAT
AGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAAACCCGACAGGACTATAA
AGATAACAGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCCTGCCGCTTACCGGATACCTG
TCCGCTTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTG
GTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTACGCCCCGACCGCTGCGCCTTATCCGGTAACCTATCGT
CTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGG
TATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATC
TGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGT
AGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTTTACCAA
TGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTG
TAGATAACTCAGTACGAGGAGGCTTACCATCTGCGCCCAAGCTGCAATGATACCCGAGACCAACGCTCACCG
GCTCCAGATTTATCAGCAATAAACCCAGCCAGCCGGAAGGCGGAGCGCAGAAGTGGTCTGCAACTTATCCGCC
TCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTT
GCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCA
AGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTCAGAAGT
AAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTTCATGCCATCCGTAAGA
TGCTTTTCTGTGACTGGTGAAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGC
CCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGTCTCATCATTTGGAAAACGTTCTTCG
GGGCGAAAACCTCAAGGATCTTACCCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTGCACCAACTGATCT
TCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGAAAAAAGGGAATA
AGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGT
CTCAT
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In **red**, *E. coli* murein lipoprotein promoter. In **bold**, (C327 to G46 of AJ536613), with the repeated hammerhead ribozyme domain **highlighted in yellow**, and their self-cleavage sites **underlined**. In **orange**, inserted between T245 and T246 of ELVd cDNA, group-I *Tetrahymena termophila* 26S rRNA intron with intron-exon permutation between T235 and C236; 10 nt of both flanking exons **underlined**. **Highlighted in green**, inverted repeat of an 83-nt fragment of

the *DvSSJ1* gene. In green, non-permuted sequence of the same intron, with the 10 nt of both flanking exons underlined. In fuchsia, *E. coli* ribosomal *rrnC* terminator. In gray, pUC replication origin. Highlighted in light grey, ampicillin resistance gene (in reverse orientation), with the promoter highlighted in dark gray).

>pLPIE-DvSSJ1 (2694 bp)

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CGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAAAAATATTCTCAACATAAAAACTTTG
TGTAATACTTGTAAACGCTGCTTCTGTTGATATGGATGCAGTTCACAGACTAAATGTCGGTCGGGGAAGATGTATT
CTTCTCATAAGATATAGTCGGACCTCTCCTTAATGGGAGCTAGCGGATGAAGTGATGCAACACTGGAGCCGCTGG
GAACTAATTTGTATGCGAAAGTATATTGATTAGTTTTGGAGTACTCGTAAGGTAGCACTTGTCTGAAATTGC
TGAAGTTGGTGATCAATTTGATATGTCTCATCTTGTACCGAACCGGATATCAAGGCTACTTCTTATGACTCTCTA
AATAGCAATATTTACCTTTGGAGGGAAAAGTTATCAGGCATGCACCTGGTAGCTAGTCTTTAAACCAATAGATTG
CATCGGTTTTAAAAGGCAAGACCGTCAAATTGCGGGAAAAGGGGTCAACAGCCGTTCAGTACCAAGTCTCAGGGGAA
ACTTTGAGATGGCCTTGCAAAGGGTATGGTAATAAGCTGACGGACATGGTCTTAACCACGCAGCCAAGTCTTAAG
TCAACAGATCTTCTGTTGATATGGATGCAGTTCACAGACTAAATGTCGGTCGGGGAAGATGTATTCTTCTCATAA
GATATAGTCGGACCTCTCCTTAATGGGAGCTAGCGGATGAAGTGATGCAACACTGGAGCCGCTGGGAACTAATTT
GTATGCGAAAGTATATTGATTAGTTTTGGAGTACTCGTAAGGTAGCAAGAAGTAGCCTTGATATCCGGTTCGGTA
CAAGATGAGACATATCAAATTGATCACCAACTTCAGCAATTTCAGGACAATGGTATGACTCTCTAAATAGCAATA
TTTACCTTTGGAGGGAAAAGTTATCAGGCATGCACCTGGTAGCTAGTCTTTAAACCAATAGATTGCATCGGTTTA
AAAGGCAAGACCGTCAAATTGCGGGAAAAGGGGTCAACAGCCGTTTCAGTACCAAGTCTCAGGGGAAACTTTGAGAT
GGCCTTGCAAAGGGTATGGTAATAAGCTGACGGACATGGTCTTAACCACGCAGCCAAGTCTTAAGTCAACAGATG
AAATCATCCTTAGCGAAAGCTAAGGATTTTTTTTATCTGAAATGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCC
CCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAAACCCGACAGGACTATAAAGATAACCAGGC
GTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCT
CCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAA
GCTGGGTGTGTGCACGAACCCCGTTACGCCGACCCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAA
CCCGGTAAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGGATGTAGGCGG
TGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCT
GAAGCCAGTTACCTTCGGAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTT
TTTTGTTTGCAAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTTACCAATGCTTAATCAG
TGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTAC
GATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGAGCCACGCTCACCGGCTCCAGATTT
ATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTATCCGCTCCATCCAGTC
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ATGATCCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTCAGAAGTAAGTTGGCCGC
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GACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCCGGCTCAAT
ACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTTGGAAAACGTTCTTCGGGGCGAAAAC
CTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACCTGATCTTCAGCATCTTT
TACTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGAAAAAAGGGAATAAGGGCGACACG
GAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTATATCAGGGTTATTGTCTCAT
```

In red, *E. coli* murein lipoprotein promoter. In orange, group-I *Tetrahymena thermophila* 26S rRNA intron with intron-exon permutation between T235 and C236; 10-nt of both flanking exons underlined. Highlighted in green, inverted repeat of an 83-nt fragment of the *DvSSJ1* gene. In green, non-permuted sequence of the same intron, with the 10 nt of both flanking exons underlined. In fuchsia, *E. coli* ribosomal *rrnC* terminator. In gray, pUC replication origin. Highlighted in light grey, ampicillin resistance gene (in reverse orientation), with the promoter highlighted in dark gray).

>p15LtRn1Sm (5415 bp)

```
CCCCGGGGGGCGGGCGGGCCGC CCGCGAAATCGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAA
AAAATATTCTCAACATAAAAACTTTGTGTAATACTTGTAAACGCTGGGAGACCACAACGGTTTCCCTCTAGAAA
TAATTTGTTTAACTTTAAGAAGGAGATATACCATGTCGGTTCGCATAGGGTCATTTACTCTTTCACTCATTAC
AAACTCTATAATCTCTCTTCTTTATCATCTTTGCTTCTAGAATCTTCTTCCCTTTCAATCTCTTCTCTT
CACACGTTCTTCACTCATGCCCAACAATCAGGAAAGGGGTGGTTATGAAGGAAAAAATGGCAAGTGAGGCCA
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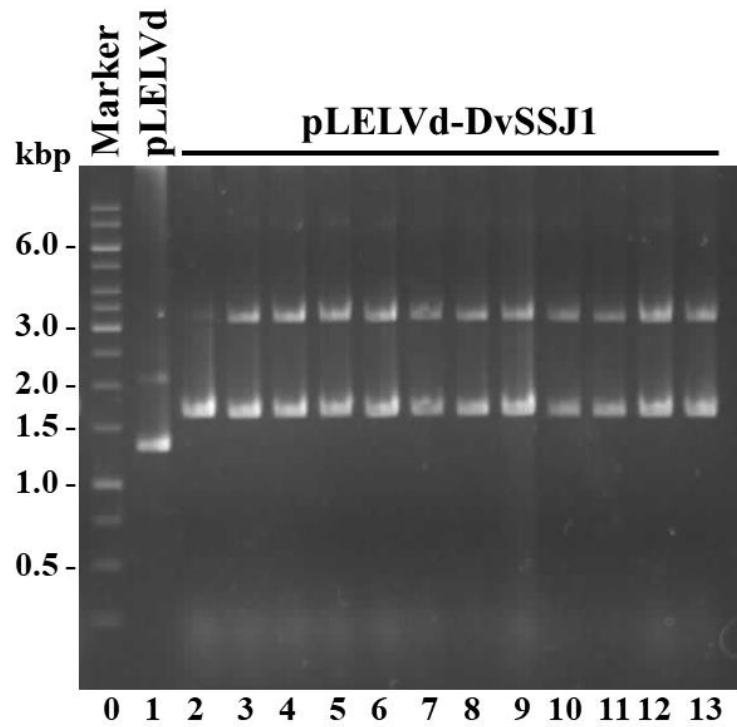
AACGTTTCAGTTTGCTCATGGAAAACGGTGTAAACAAGGGTGAACACTATCCCATATCACCAGCTCACCGTCTTTC  
 ATTGCCATACGGAATTCGGGATGAGCATTTCATCAGGCGGGCAAGAATGTGAATAAAGGCCGATAAACTTGTGC  
 TTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACT  
 GACTGAAATGCCTCAAATGTTCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTTC  
 TCCATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTTCATTA  
 TGGTGAAAGTTGGAA

In **red**, *E. coli* murein lipoprotein promoter. In **bold**, chloroplastic isoform of the eggplant tRNA ligase (JX0225157), with the theoretical amino-terminal transit peptide **highlighted in green**, the carboxyl terminal hexahistidine tag in **blue** and the start and stop codons **underlined**. In **fuchsia**, T7 bacteriophage terminator. In gray, p15A replication origin. **Highlighted in light grey**, chloramphenicol resistance gene (in reverse orientation), with the promoter **highlighted in dark gray**).

>p15CAT (1634 bp)

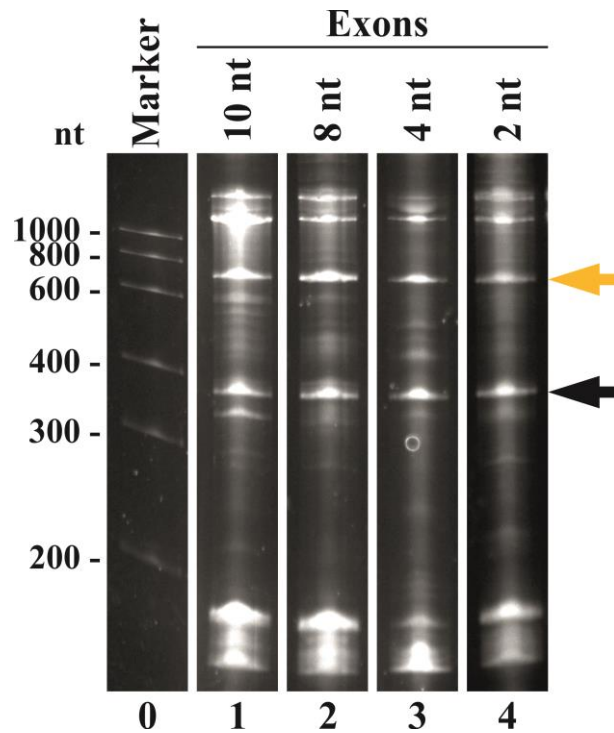
**CCCCGGGGCGGGCGGCCGCGGGCGGACGTCGGCGCCTAAGGGGCGAGATCTGGC**GGGGCCCGCGCTAGCGGAGTGTA  
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 GGTGCGTCAGCAGAATATGTGATACAGGATATATTCCGCTTCCTCGCTCACTGACTCGCTACGCTCGGTCTGTTCCG  
 ACTGCGGCGAGCGGAAATGGCTTACGAACGGGGCGGAGATTTCTGGAAGATGCCAGGAAGATACTTAACAGGGA  
 AGTGAGAGGGCGCGGCAAGCCGTTTTTCCATAGGCTCCGCCCCCTGACAAGCATCACGAAATCTGACGCTCA  
 AATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGCGGCTCCCTCGTGCCTCT  
 CCTGTTCTGCCTTTTCGGTTTACCGGTGTCATTCCGCTGTTATGGCCGCGTTTGTCTCATTCCACGCTGACACT  
 CAGTTCGGGTAGGCAGTTTCGCTCCAAGCTGGACTGTATGCACGAACCCCCCGTTTCAGTCCGACCGCTGCGCCTT  
 ATCCGGTAACTATCGTCTTGAGTCCAACCCGAAAAGACATGCAAAAGCACCCTGGCAGCAGCCACTGGTAATTG  
 ATTTAGAGGAGTTAGTCTTGAAGTCATGCGCCGGTTAAGGCTAAACTGAAAGGACAAGTTTTGGTGACTGCGCTC  
 CTCCAAGCCAGTTACCTCGGTTCAAAGAGTTGGTAGCTCAGAGAACCCTTCGAAAAACCGCCCTGCAAGGCGGTTT  
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 CCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAGACGGCATGAT  
 GAACCTGAATCGCCAGCGGCATCAGCACCTTGTGCTTGGTATAATATTTGCCCATGGTGAAAACGGGGCGA  
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 TATTCTCAATAAACCTTTAGGGAAATAGGCCAGTTTTACCGTAAACACGCCACATCTTGCGAATATATGTGTA  
 GAAACTGCCGAAATCGTCTGGTATTCACTCCAGAGCGATGAAAACGTTTTAGTTTTGCTCATGGAAAACGGTGT  
 AACAAGGGTGAACACTATCCCATATCACCAGCTCACCGTCTTTCATTGCCATACGGAATTCGGGATGAGCATTCA  
 TCAGGCGGGCAAGAATGTGAATAAAGGCCGATAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCG  
 TAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAATGTTCTTTACGAT  
 GCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCTCCATTTTAGCTTCCTTAGCTCCTGAAAATC  
 TCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTTCATTATGGTGAAAGTTGGAA

In gray, p15A replication origin. **Highlighted in light grey**, chloramphenicol resistance gene (in reverse orientation), with the promoter **highlighted in dark gray**). **Highlighted in yellow**, polylinker with recognition sites for NotI (**underlined**) and BglII (**underlined**).

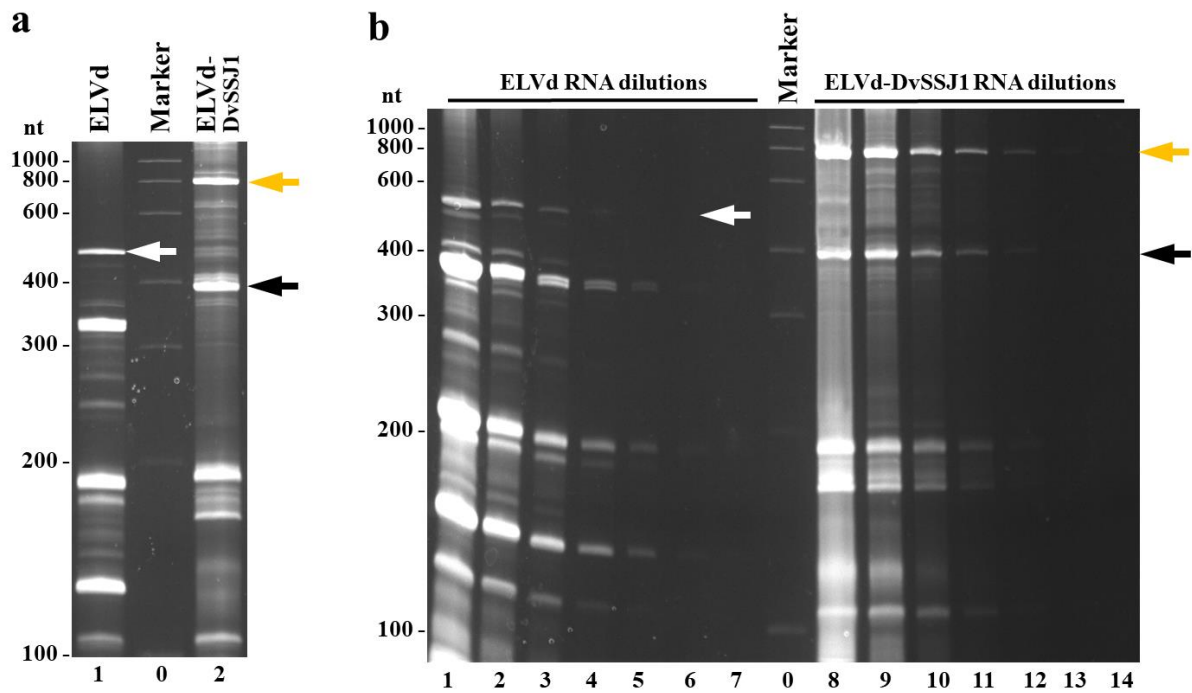


**Supplemental Figure S1.** Construction of expression plasmids to produce *DvSSJ1*-derived dsRNA in *E. coli*. Plasmids purified from independent *E. coli* clones were separated by electrophoresis through an agarose gel, which was stained with ethidium bromide. Lane 0, DNA marker ladder with some of the sizes in bp on the left; lane 1, control plasmid pLELVd expressing an empty ELVd; lanes 2 to 13, plasmids pLELVd-DvSSJ1 to express the *DvSSJ1*-derived dsRNA on an ELVd scaffold obtained from 12 independent *E. coli* colonies.

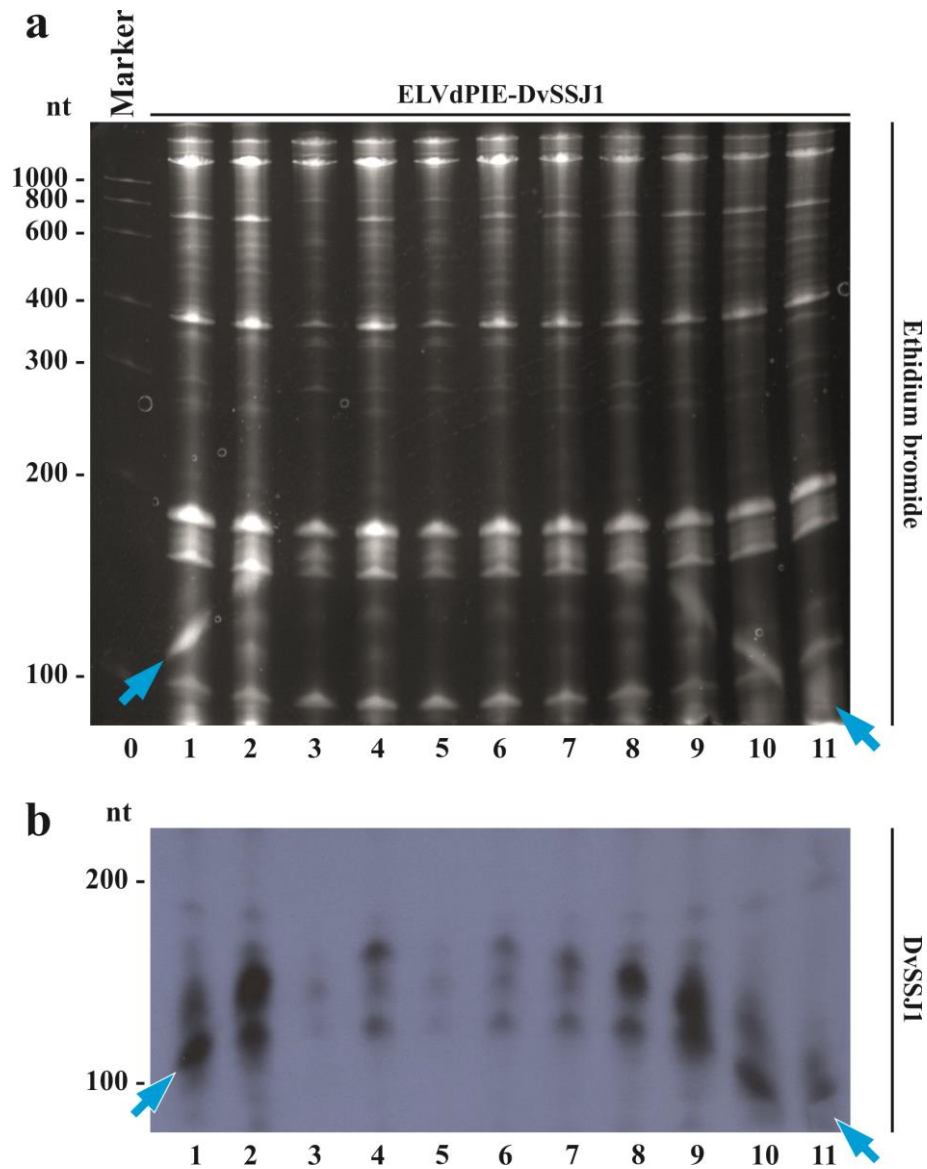




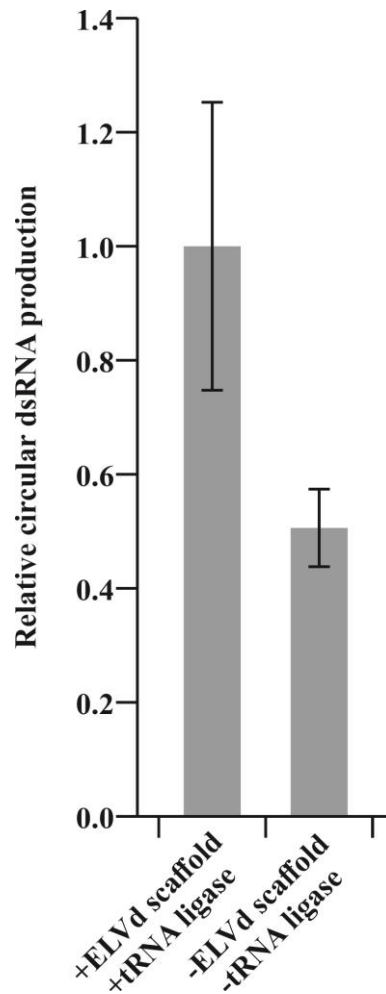
**Supplemental Figure S2.** Effect of exon size in *T. thermophila* intron processing in the ELVd-based system to produce dsRNA in *E. coli*. Aliquots of RNA preparations from *E. coli* clones cotransformed with p15LtRnlSm and a series of pLELVd derivatives to produce a 100-bp dsRNA, in which the exons that flank the *T. thermophila* intron are increasingly shorter, as indicated, were separated by denaturing PAGE. The gel was stained with ethidium bromide. Lane 0, RNA marker with sizes (in nt) on the left; lanes 1 to 4, RNAs from constructs with 10, 8, 4 and 2-nt exons, respectively. Orange and black arrows point the positions of the recombinant ELVd-dsRNA and the spliced introns, respectively.



**Supplemental Figure S3.** Large scale RNA preparations produced in *E. coli* by means of the viroid-based system and used in the WCR bioassay. RNAs were separated by denaturing PAGE and the gels stained with ethidium bromide. (a and b) Lane 0, RNA marker ladder with sizes in nt on the left. (a) Lanes 1 and 2, large-scale RNA preparations from *E. coli* transformed with p15LtRnlSm and pLELVd or pLELVd-DvSSJ1, respectively. (b) Dilution analysis of the ELVd (lanes 1 to 7) and the ELVd-DvSSJ1 (lanes 8 to 14) RNA preparations. White, orange and black arrows point to ELVd, ELVd-DvSSJ1 and spliced-intron RNAs, respectively.



**Supplemental Figure S4.** Analysis of the recombinant circular dsRNA. RNA preparations from different *E. coli* clones (lanes 1 to 11) co-transformed with p15LtRnlSm and pLELVdPIE-DvSSJ1 were separated by denaturing PAGE. The gel was (a) stained with ethidium bromide and (b) the RNA transferred to a membrane and hybridized with a  $^{32}\text{P}$ -labelled probe to detect DvSSJ1 RNA. Lane 0, RNA marker with sizes in nt on the left. Blue arrows point to the recombinant circular dsRNA that exhibits an inverted smile migration across the gel.



**Supplemental Figure S5.** Effect of the the ELVd scaffold and the tRNA ligase on accumulation of a recombinant circular dsRNA. RNA preparations from *E. coli* clones cotransformed with p15LtRnlSm and a pLELVdPIE-derivative to produce a 100-bp dsRNA or the empty ligase plasmid (p15CAT) and a pLPIE-derivative (no ELVd scaffold) to produce the same 100 bp dsRNA were separated by denaturing PAGE. After staining the gels with ethidium bromide, recombinant circular dsRNA accumulation was quantified (in fluorescence arbitrary units) using an image analyzer. Normalized average fluorescence is plotted. Error bars represent standard deviation (n = 5).

**Supplemental Table S1.** Primers used in the PCR amplifications to build expression plasmids pLELVd-DvSSJ1, pLELVdPIE-DvSSJ1 and pLPIE-DvSSJ1.

<b>Name</b>	<b>Sequence (5' to 3')</b>
D2623	ACCATTGTCCTGAAATTGCTGAAGTTGGTGATCAATTTGATATGTCTCA
D2624	AAGAAGTAGCCTTGATATCCGGTTCGGTACAAGATGAGACATATCAAAT
D2625	CCTCTCCCCCTCCCAGGTAATATCCCCTTACCATTGTCCTGAAATTG
D2626	TTTAGAGAGTCATAAGAAGTAGCCTTGATATCCG
D2627	AAGGCTACTTCTTATGACTCTCTAAATAGCAATATTTAC
D2628	AAGGCTACTTCTTGGCTACCTTACGAGTACTCC
D2629	TCGTAAGGTAGCCAAGAAGTAGCCTTGATATCCG
D2630	ACCCACCCTCCTAGGGAACACATCCTTGAACCATTGTCCTGAAATTG
D2936	AAGGGGATAGTACCTGGGAG
D2937	ACCATTGTCCTGAAATTGCTG
D2940	ACCATTGTCCTGAAATTG
D2941	TCAAGGATGTGTTCCCTAG
D3606	CTTCTGTTGATATGGATG
D3285	CAGCGTTACAAGTATTACAC
D3607	GAAATCATCCTTAGCGAAAGC
D3608	ATCTGTTGACTTAGGACTTGGC