

SUPPLEMENTARY INFORMATION

A viroid-derived system to produce large amounts of recombinant RNA in *Escherichia coli*

José-Antonio Daròs*, Verónica Aragonés & Teresa Cordero

Instituto de Biología Molecular y Celular de Plantas (Consejo Superior de Investigaciones Científicas-Universitat Politècnica de València), 46022 Valencia, Spain. Correspondence and requests for materials should be addressed to J.-A.D. (email: jadaros@ibmcp.upv.es)

Figure S1. Nucleotide sequences and elements of plasmids pLELVd, p15tRn1Sm and p15mCherry.

>pLELVd (2050 bp)

```

CGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAAAAATATTCTCAACATAAAAACTTTG
TGTAATACTTGTAAACGCTGCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAAGGTCGAAATGGGGTTT
CGCCATGGGTGCGGACTTTAAATTCGGAGGATTTCGTCTTTAAACGTTCTTCCAAGAGTCCCTTCCCCAAACCCCT
TACTTTGTAAGTGTGGTTCGGCGAATGTACCGTTTCGTCTTTTCGGACTCATCAGGGAAAAGTACACACTTTCCGA
CGGTGGGTTTCGTGACACCTCTCCCCCTCCAGGTAATATCCCTTTCAAGGATGTGTTCCCTAGGAGGGTGGGT
GTACCTCTTTTGGATTGCTCCGGCCTTCCAGGAGAGATAGAGGACGACCTCTCCCATAGGGTGGTGTGTGCCAC
CCCTGATGAGACCGAAAAGGTCGAAATGGGGGAAATCATCTTAGCGAAAGCTAAGGATTTTTTTTATCTGAATG
CGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGC
GAAACCCGACAGGACTATAAAGATAACCAGGCGTTTTCCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCGGACC
TGCCGCTTACCGGATACCTGTCCGCTTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGT
ATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCCAGCCGACCGCTGCG
CCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTA
ACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCCTGAAGTGGTGGCTAACTACGGCTACACTA
GAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCG
GCAAAACAAACCCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTC
AAGAAGATCCTTTTTACCAATGCTTAATCAGTGAGGACCTATCTCAGCGATCTGTCTATTTCGTTCAATCCATAG
TTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTCTGCATCCATAG
CGCGAGAGCCACGCTCACCAGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTG
GTCCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTA
ATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCA
GCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTC
CTCCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTATGGCAGCACTGCATAATTTCTCTTA
CTGTCATGCCATCCGTAAGATGCTTTTTCTGTGACTGGTGAAGTACTCAACCAAGTCATTTCTGAGAATAGTGTATGC
GGCGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCA
TCATTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCCTGTTGAGATCCAGTTCGATGTAACCA
CTCGTGACCCAACTGATCTTACGATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAA
ATGCCGCAAAAAGGGAATAAGGGCGACACGAAATGTTGAATACTCATACTCTTCTTTTTTCAATATTTATGAA
GCATTTATCAGGGTTATTGTCTCAT

```

E. coli **murein lipoprotein promoter** is in red and **ELVd** cDNA (C327 to G46 of AJ536613) is in black with the repeated hammerhead ribozyme domain on yellow background. Ribozymes self-cleavage sites are underlined. *E. coli* ribosomal **rrnC terminator** is in fuchsia. **pUC replication origin** is in gray and **ampicillin resistance gene** (inverse orientation) on gray background (**promoter** on dark gray background).

>p15tRn1Sm (5338 bp)

```

TAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATA
TACCATGTCGGTTTTCGCATAGGGTCATTTACTCTTTTCACTCATTACAACTCTATAATCTCTCTTCTTTTATC
ATCTTTGCCTTCTAGAATCTTCTTCCCTTTTCAATCTCCTTCTTTCACACGTTCTCTTCACTCATGCCAACAA
TCAGGAAAGGGGTGGTTATGAAGGAAAAAATGGCAAGTGAGGCCAAGTTCCAATAGGGTACCAGGCTCGTCTTC
AAATGTGGAACCTGTATCTGCTGCAACTGCTGAAGCCATTACCGACCGTCTAAAAGTCCGTGGATATTACTGAAAG
TGGTGCACAGTCTAGTGTTCAGTTCACATCTCTTTCAGTTTTGGCAGCGTTGGATTAGCACCCCAGTCACCTGTGCA
ACATCAAAAAGTAATCTGGAAACCCAAATCATATGGAACAGTGTCTGGAGCCCAGTGGTTGAAGCTGGAAAAAC
ACCAGTTGAACAAAAAAGTGCTCTTTTAAAGTAAATTTATTCAAGGGTAATTTATTGGAAAAATTTACTGTAGATAA
CTCAACATTTCTCGAGAGCCCAAGTAAGGGCCACTTTCTACCCAAAATTTGAGAATGAGAAATCAGATCAGGAGAT
CAGGACAAGGATGATAGAGATGGTCTCCAAAGGCTTGGCTATAGTCGAGGTCACACTTAAGCATTCTGGATCTCT
TTTTATGTATGCTGGGCATGAAGGTGGAGCATATGCCAAGAATAGCTTTCGGGAATATCTATACTGCCGTTGGCGT
CTTTGTTCTTGGACGGATGTTTTCGTGAGGCATGGGGAACATAAAGCAAGCAAGAAGCAAGCAGAGTTCAATGAGTT
TCTTGAGCGCAATCGTATGTGCATATCAATGGAGTTGGTCACGGCAGTGTGGGGGACCACGGACAACGCCCACG
AGATGATTATGCGGTTGTGACTGCAGTCACGGAGTTGGGAAATGGAAAACCAACTTTCTATTCAACTCCCAGATGT
AATTGCTTTTTGTCAGGGAATGGCGATTACCAACAAATCATGTATGGCTGTCTCAACAAGGAAATCAGTGACTTC
CTTCTTTGCTGCGTATGATGCACTTTGCGAGGAAGGTACAGCAACCACCGTTTTCGAGGGCTCTCAGCGAAGTTGC
TGATATTTCTGTACCTGGATCAAAAAGACCATATAAAGTGCAGGGTGAAATTTTGGAGGGTCTCGTGGCCCGCAT
CGTAAAACGTGAGAGCTCAGAGCATATGGAGCGGGTCTGAGAGATTTTCTCCTCCGCCATCAGAGGGTGAGGG
TTTTGGACCTGGGACCTACGTTACGTGAAATTTGTGCTGCAAAACAGATCAGAAAAGCAGCAAAATAAAGGCACCTTCT

```

TCAGAGTGCTGGCACGGCTTTCTGCCCGAATTATTTGGACTGGTTTTGGAGATGAAAACCTCTGGTTCACATTC AAG
AAATGCTGATCGATCTGTTGTCTCAAAGTTCTTACAATCACATCCTGCTGATCTTTATACAGGAAAAATACAGGA
AATGGTTTCGCTTGATGAGGGAAAAGCGCTTTTCTGCTGCTTTTCAAGTGTCAATTATACTTACATAAAAAATTAATGA
TGTATCGAGTAACAACCTGCCTTTCAAATGGTGATCCATGTATATAGTGATTCAGGCTTCCGCCGGTACCAGAA
AGAGATGAGGCACAAACCAGGACTATGGCCTTTGTATCGAGGCTTTTTTGTGACCTGGATTTATTC AAGGTCAA
TGAGAAGAAAACCTGCTGAAATGGCAGGAAGCAACAATCAAATGGTAAAAAATGTGGAAGAGGACAACAGTTTAGC
TGATGAAGATGCAAATCTGATGGTCAAGATGAAATTTCTTACTTACAAGTTGAGAACTTTTTTGATCCGTAATGG
CTTGTGCGACTCTTTCAAAGAAGGACCTTCTGCGTATAAGTCTTATTACCTGAGGCAAATGAAAATTTGGAATAC
TTCAGCAGCCAAGCAACGAGAACTCAGCAAGATGCTTGATGAATGGGCAGTATATATACGCAGAAAAATATGGGAA
CAAACCATTGTCATCATCCACATACCTAAGTGAAGCTGAGCCTTTTCTTGAACAATATGCAAAGCGTAGTCCACA
AAATCATGCTTTGATAGGATCTGCTGGAAAATTTGTCAAAGTTGAAGATTTTCATGGCTATTTGTTGAAGGAGAAGA
TGAAGAGGGTGATCTCGAGCCTGCGAAAAGATATTGCTCCTTCAAGCCCTAGTATTTCCACCAGAGACATGGTGGC
AAAGAATGAGGGTCTCATTATTTTTCTTTCCAGGAATACCAGGTTGTGCTAAAATCTGC ACTTTGTAAGGAAAATACT
GAATGCTCCAGGAGGGCTTGGAGATGATCGACCAGTTAACAGTTTAAATGGGTGATCTTATTAAGGTAGATATTG
GCAAAAAGTTGCTGATGAACGTCGAAGAAAACCTTACTCGATCATGCTTGCTGACAAGAAATGCACCAAATGAGGA
AGTATGAAAACAAATGAGAACATGTGCCTAAGCACCAGGAGCATCTGCTATTCCAGTTATACCTGATTCAGAAG
AACTGAAACTAATCCATTCTCTATTGATGC ACTTTGCGGTTTTTATATTCCGAGTACTTCACCGTGTCAATCATCC
GGGAAATCTTGACAAGTCATCTCAAATGCTGGATATGTGATGCTTATGTTTTATCACCTTTATGATGGAAGAG
CCGTCCAGGAGTTCGAGAGTGAGCTTATTGAACGTTTTGGATCGCTTGTGAGAATTCCTGTACTGAAACCTGAGAG
GTCTCCTCTTCCGATTCTGTGAGGTCTATTATCGAGGAGGGACTCAGTCTGTACAGACTTCATACAACGAAACA
TGGAAGATTGGAGTCTACAAAAGGGACATATGTACAAGAGTGGGTAAATGGGAGAAAGCAATTGAGAGATATTCT
ACTTGGAAATGCAGACTATCTCAATTC AATACAGGTTCCATTTGAATTTGCCGTTAAAAGAAAGTCCTTGAAACACT
GAAAGTTATTGCGAGGGGGCGAATATGCAGTGCCTGCTGAGAAGAGGAAGCTAGGATCCATTGTATTCGCCGCTAT
CAGCCTGCCAGTTCAGAAATCTAGGTCTTCTAATGATCTAGCAAAGAAAGATCCAAAGGTTGGCGATTTTCAT
TAAGACAAGAGCATGGAGAGCAGCATTGAGAAGGCCCATCTTACCCTGGCTCACAAGAGAAGTACCGGTGTAC
TGCAGTTGCCAATTACGGTTCTTTCTTTCATCAAAGGTTGCCAGTAGACGTGGCTGCTTTGTTCTTCCGATAA
ATTGGCTGCACTAGAAGCTGAGCCTGGCTCTGTTGAAGGTGAAAAGATCAATTTCTAAAACCTGATGGCCCCATAT
CACATTATGGTCTGGTGCAGGAGTTGCCGCAAAGATGCCAATACACTACCACAGTTACTTTCCCAAGGGGAAGGC
TACCCGCATTGATATAAATCCACCGGTCACTATAACTGGCACTCTCGAATTCTTT**CACCACCACCACCACCAC**TG
AGATCCGGCTGCTAACAAAGCCCGAAAAGGAAGCTGAGTTGGCTGCTGCCACCCTGAGCAATAAC**TAGCATAACC**
CCTTGGGGCTCTAAACGGGTCTTGAGGGGTTTTTGCTGAAAGG**AGATCTGGCGGGGCC**CGCTAGCGGAGTG
TATACTGGCTTACTATGTTGGCACTGATGAGGGTGTGAGTGAAGTGCTTCATGTGGCAGGAGAAAAAGGCTGCA
CCGGTGCCTCAGCAGAATATGTGATACAGGATATATTCCGCTTCTCGCTCACTGACTCGCTACGCTCGGTGCTT
CGACTGCGGCGAGCGGAAATGGCTTACGAACGGGGCGGAGATTTCTGGAAGATGCCAGGAAGATACTTAACAGG
GAAGTGAGAGGGCCGCGGCAAAGCCGTTTTTCCATAGGCTCCGCCCCCTGACAAGCATCACGAAATCTGACGCT
CAAATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCCTGGCGGCTCCCTCGTGCCT
CTCCTGTTCTGCTTTCCGTTTTACCGGTGTCAATCCGCTGTTATGGCCGCGTTTTGTCTCATTCCACGCTGACA
CTCAGTTCCGGGTAGGCAGTTCCGCTCCAAGCTGGACTGTATGCACGAACCCCCCGTTTCCAGTCCGACCGCTGCGCC
TTATCCGGTAACTATCGTCTTGAGTCCAACCCGAAAAGACATGCAAAAAGCACCCTGGCAGCAGCCACTGGTAAT
TGATTTAGAGGAGTTAGTCTTGAAGTCATGCGCCGGTTAAGGCTAAACTGAAAAGGACAAGTTTTGGTGACTGCGC
TCCTCCAAGCCAGTTACCTCGGTTCAAAGAGTTGGTAGCTCAGAGAACCTTCGAAAAACCGCCCTGCAAGGCGGT
TTTTTCGTTTTTCAGAGCAAGAGATTACGCGCAGACCAAAACGATCTCAAGAAGATCATCTTATTAAT**TTACGCCCC**
GCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAGACGGCATG
ATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTGCGCTTGCATATAATTTGCCATGGTGAAAACGGGGC
GAAGAAGTTGTCCATATTGGCCACGTTTAAATCAAACCTGGTGAACACTCACCCAGGATGGCTGAGACGAAAAA
CATATTCTCAATAAACCTTTAGGGAAATAGGCCAGTTTTTACCCTAACACGCCACATCTTGCGAATATATGTG
TAGAAACTGCCGAAATCGTCTGTTTCACTCCAGAGCGATGAAAACGTTTCCAGTTTGTCTCATGGAAAACGGT
GTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCCTTTTCAATGCCATACGGAATTCGGATGAGCATT
CATCAGGCGGGCAAGAATGTGAATAAAGGCCGATAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGC
CGTAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAATGTTCTTTACG
ATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCTCCATTTAGCTTCTTAGCTCCTGAAAA
TCTCGATAACTCAAAAATACGCCCGGTAGTGATCTTATTTCAATATGGTGAAGTTGGAACCCGGGGCGGCGG****
CCGCCCGCGAAAT

T7 bacteriophage promoter underlined with the **+1** nucleotide of the transcript on yellow background. Eggplant tRNA ligase (JX0225157) in black with the theoretical amino-terminal **transit peptide** on green background and the carboxy-terminal **hexahistidine tag** in blue. **Stop codon** in red. **T7 bacteriophage terminator** on red background. **p15A replication**

origin in gray and chloramphenicol resistance gene (reverse orientation) in blue (*promoter* in italics).

>p15mCherry (2623 bp)

TAATACGACTCACTATAGGGGGACCACAACGGTTTCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATA
TACC**ATGGTTAGCAAAGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGA**
GGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCAGCCAGACCCG
CAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTC
CAAGGCCTACGTGAAGCACCCCGCCGACATCCCGACTACTTGAAGCTGTCTTCCCCGAGGGCTTCAAGTGGGA
GCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCAT
CTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGA
GGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGA
CGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAA
CGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAAACAGTACGAACGCGCCGAGGG
CCGCCACTCCACCGGCGGCATGGATGAGCTGTATAAGAGCGCATGGAGT**CATCCTCAATTGAGAAA**GGTGGAGG
TTCTGGCGGTGGATCGGGAGGTT**CAGCGTGGAGCCACCCGAGTTCGAAAAATCCGGA**TGAGATCCGGCTGTAA
CAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAAC**TAGCATAACCCCTTGGGGCTCTAA**
ACGGTCTTGGGGTTTTTTCCTGAAAGGAGATCTGGCGGGGCCGCGCTAGCGGAGTGTATACTGGCTTACTA
TGTGGCACTGATGAGGGTGTCAAGTGAAGTGCCTCATGTGGCAGGAGAAAAAAGGCTGCACCGGTGCGTCAGCAG
AATATGTGATACAGGATATATTTCCGCTTCTCCTCGCTCACTGACTCGCTACGCTCGGTGCTGACTGCGGCGAGCG
GAAATGGCTTACGAACGGGGCGGAGATTTCTGGAAGATGCCAGGAAGATACTTAACAGGGAAGTGAAGGGGCCG
CGGCAAAGCCGTTTTTCCATAGGCTCCGCCCCCTGACAAGCATCACGAAATCTGACGCTCAAAATCAGTGGTGGC
GAAACCCGACAGGACTATAAAGATAACCAGGCGTTTTCCCCCTGGCGGCTCCCTCGTGCGCTCTCCTGTTCCTGCCT
TTCGGTTTTACCGGTGTCATTCCGCTGTTATGGCCGCGTTTTGTCTCATTCCACGCTGACACTCAGTTCGGGTAG
GCAGTTCGCTCCAAGCTGGACTGTATGCAAGAACCCCGTTAGTCCGACCGCTGCGCCTTATCCGGTAACTAT
CGTCTTGAGTCCAACCCGAAAGACATGCAAAAGCACCACTGGCAGCAGCCACTGGTAATTGATTTAGAGGAGTT
AGTCTTGAAGTCATGCGCCGGTTAAGGCTAAACTGAAAGGACAAGTTTTTGGTACTGCGCTCCTCAAGCCAGTT
ACCTCGGTTCAAAGAGTTGGTAGCTCAGAGAACCTTCGAAAAACCGCCCTGCAAGGCGGTTTTTTCGTTTTTCA
GCAAGAGATTACGCGCAGACCAAAACGATCTCAAGAAGATCATCTTATTAATTACGCCCGCCCTGCCACTCATC
GCAGTACTGTTGTAATTCATTAAGCATTTCTGCCGACATGGAAGCCATCACAGACGGCATGATGAACCTGAATCGC
CAGCGGCATCAGCACCTTGTGCGCTTGCCTATAATATTTGCCCATGGTGAACCGGGGGCGAAGAAGTTGTCCAT
ATTGGCCACGTTTTAAATCAAACCTGGTGAACCTCACCCAGGGATTGGCTGAGACGAAAAACATATTTCTCAATAAA
CCCTTTAGGGAAATAGGCCAGGTTTTACCGTAACACGCCACATCTTGCGAATATATGTGTAGAAACTGCCGGAA
ATCGTCTGGTATTCACTCCAGAGCGATGAAAACGTTTTAGTTTTGCTCATGGAAAACGGTGTAAACAAGGGTGAAC
ACTATCCCATATCACAGCTCACCGTCTTTTATTGCCATACGGAATTCCGGATGAGCATTCATCAGGCGGGCAAG
AATGTGAATAAAGGCCGGATAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTG
AACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAATGTTCTTTACGATGCCATTGGGATAT
ATCAACGGTGGTATATCCAGTGATTTTTTTCTCCATTTAGCTTCTTAGCTCCTGAAAATCTCGATAACTCAA
AAATACGCCCGGTAGTGATCTTATTTCAATTATGGTGAAGTTGGAACCCGGGGCGGCGGCCGCCCGCAAT

T7 bacteriophage promoter underlined with the **+1** nucleotide of the transcript on yellow background. **mCherry** (AY678264) cDNA on red background, including four **silent mutations** (underlined) and a carboxy-terminal **Twin-Srep-tag** in red. **Stop codon** in red. **T7 bacteriophage terminator** on red background. p15A replication origin in gray and chloramphenicol resistance gene (reverse orientation) in blue (*promoter* in italics).

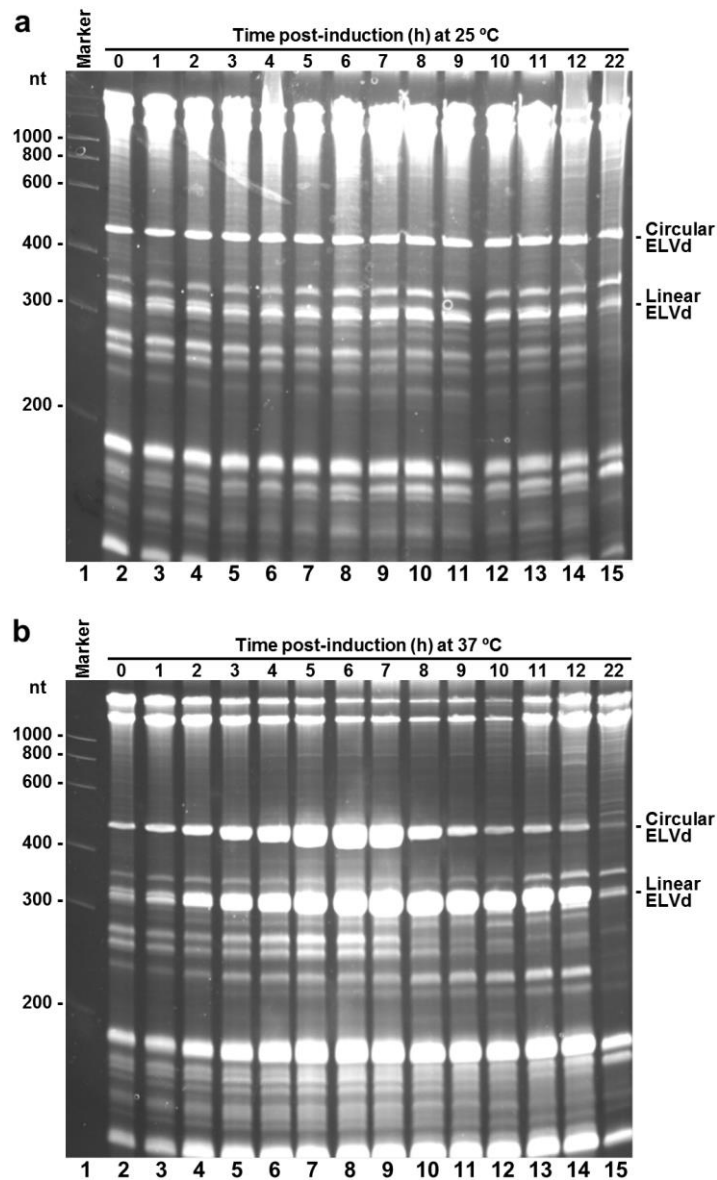


Figure S2. Time-course analysis of the RNA that accumulated in *E. coli* cells that co-expressed ELVd RNA and eggplant tRNA ligase, grown at 25°C (a) and 37°C (b). Aliquots of the cultures were taken at the indicated time points after induction of tRNA ligase expression. RNA was extracted and analyzed by denaturing PAGE and ethidium bromide staining. Lanes 1, RNA marker with the sizes of the standards indicated on the left in nt; lanes 2 to 15, RNA from aliquots of the cultures taken at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 22 h post-induction. The positions of the circular and linear ELVd RNAs are indicated on the right. *E. coli* cultures were induced with 0.4 mM IPTG at DO₆₀₀ 0.6. Each lane contains an aliquot of RNA corresponding to 0.8 ml of culture.

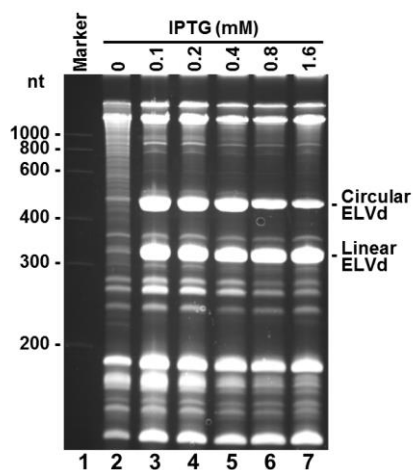


Figure S3. Analysis of the RNA that accumulated in *E. coli* cells that co-expressed ELVd RNA and eggplant tRNA ligase, induced with different amounts of IPTG. RNA preparations were separated by denaturing PAGE and the gel stained with ethidium bromide. Lane 1, RNA marker with the sizes of the standards indicated on the left in nt; lane 2, RNA from a non-induced culture; lanes 3 to 7, RNAs from cultures induced with 0.1, 0.2, 0.4, 0.8 and 1.6 mM IPTG, respectively. The positions of the circular and linear ELVd RNAs are indicated on the right. *E. coli* cultures were grown at 37°C and induced at DO₆₀₀ 0.6. Bacteria were harvested at 6 h post-induction. Each lane contains an aliquot of RNA corresponding to 0.8 ml of culture.

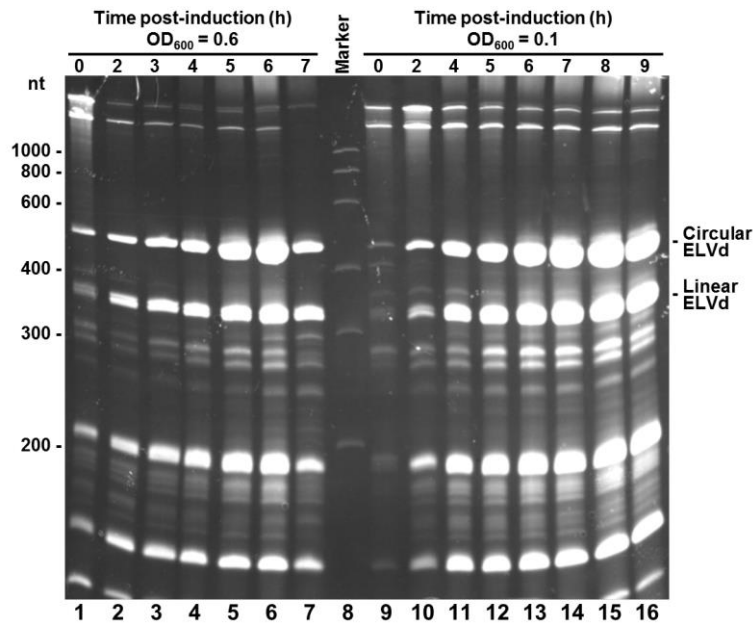


Figure S4. Time-course analysis of the RNA that accumulated in *E. coli* cells that co-expressed ELVd RNA and eggplant tRNA ligase, induced at different OD_{600} . Aliquots were taken from the cultures at different time points, and RNA extracted and analyzed by denaturing PAGE and ethidium bromide staining. Lanes 1 to 7, RNAs from the culture induced at OD_{600} 0.6 taken at 0, 2, 3, 4, 5, 6 and 7 h post-induction; lane 8, RNA marker with the sizes of the standards indicated on the left in nt; lanes 9 to 16, RNAs from the culture induced at OD_{600} 0.1 taken at 0, 2, 4, 5, 6, 7, 8 and 9 h post-induction. The positions of the circular and linear ELVd RNAs are indicated on the right. *E. coli* cultures were grown at 37°C and induced with 0.1 mM ITPG. Each lane contains an aliquot of RNA corresponding to 0.4 ml of culture.

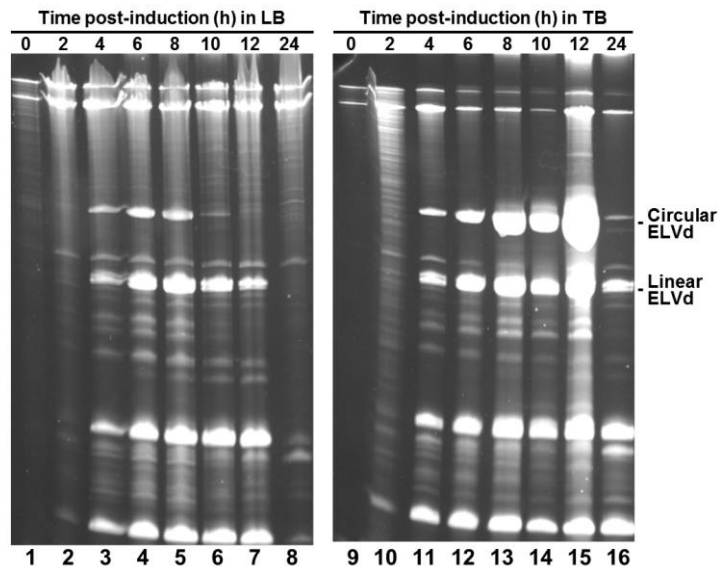


Figure S5. Time-course analysis of the RNA that accumulated in *E. coli* cells that co-expressed ELVd RNA and eggplant tRNA ligase, grown in LB and TB media. Aliquots were taken from the cultures at different time points, as indicated. RNAs were extracted and analyzed by denaturing PAGE and ethidium bromide staining of the gels. Lanes 1 to 8, RNAs from the culture grown in LB medium taken at 0, 2, 4, 6, 8, 10, 12 and 24 h post-induction; lanes 9 to 16, RNAs from the culture grown in TB medium taken at 0, 2, 4, 6, 8, 10, 12 and 24 h post-induction. The positions of the circular and linear ELVd RNAs are indicated on the right. *E. coli* cultures were grown at 37°C and induced with 0.1 mM IPTG at DO₆₀₀ 0.1 mM. Each lane contains an aliquot of RNA corresponding to 0.4 ml of culture.

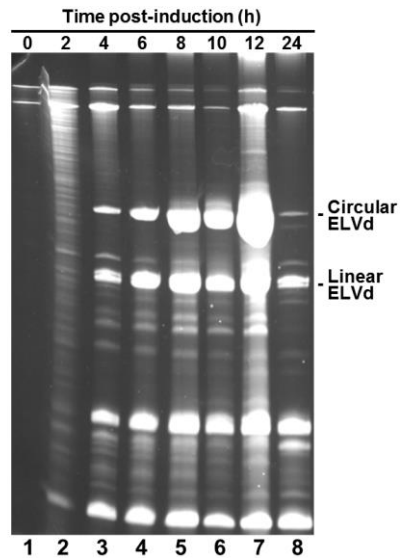


Figure S6. Time-course analysis of the RNA accumulated in *E. coli* cells that co-expressed ELVd RNA and eggplant tRNA ligase in optimized conditions (culture grown at 37°C in TB medium and induced with 0.1 mM ITPG at DO_{600} 0.1). Aliquots from the culture were taken at different time points and RNA purified. RNAs were separated by denaturing PAGE and the gel stained with ethidium bromide. Lanes 1 to 8, RNAs from aliquots taken at 0, 2, 4, 6, 8, 10, 12 and 24 h post-induction of tRNA ligase expression. The positions of the circular and linear ELVd RNAs are indicated on the right. Each lane contains an aliquot of RNA corresponding to 0.4 ml of culture.

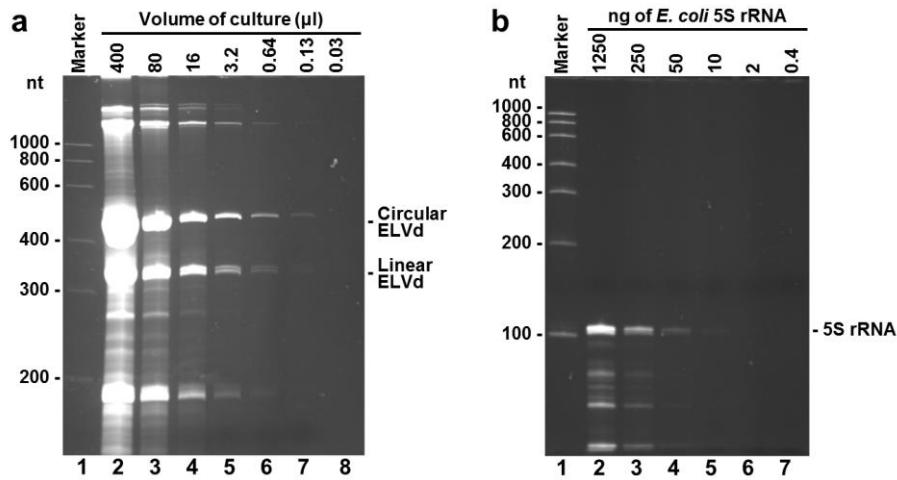


Figure S7. Quantification of the ELVd RNA produced in *E. coli* with the viroid-derived system in optimal conditions. RNA samples were separated by denaturing PAGE and the gels stained with ethidium bromide. **(a)** The RNA extracted from an aliquot of a culture taken at 12 h post-induction (see [Supplementary Fig. S6](#)) was subjected to 1/5 serial dilutions and electrophoresed. Lane 1, RNA marker with the sizes of the standards indicated on the left in nt; lanes 2 to 8, serial dilutions of the RNA preparation that corresponded to 400, 80, 16, 3.2, 0.64, 0.13 and 0.03 μl of the original culture, respectively. **(b)** A quantified preparation of *E. coli* 5S rRNA was subjected to 1/5 serial dilution before separation by PAGE and staining of the gel. Lane 1, RNA marker with the sizes of the standards indicated on the left in nt; lanes 2 to 7, 1250, 250, 50, 10, 2 and 0.4 ng of the *E. coli* 5S rRNA standard, respectively. The positions of the circular and linear ELVd RNAs, and the 5S rRNA are indicated on the right of the corresponding panel.

Figure S8. Sequences of the full-length ELVd cDNA present in plasmid pLELVd and the different ELVd deleted forms assayed in the viroid-derived system to produce recombinant RNA in *E. coli*. The position of the deletion is indicated on blue background. Hammerhead ribozyme domain is indicated on yellow background.

>ELVd

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATGGGTCTGGGACTTTAAATTCG
GAGGATTCGTCTTTAAACGTTCCCTCCAAGAGTCCCTTCCCCAAACCCCTTACTTTGTAAGTGTGGTTCGGCGAAT
GTACCGTTTTCGTCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGTGACACCTCTCCCC
CTCCCAGGTACTATCCCCTTTCAAGGATGTGTTCCCTAGGAGGGTGGGTGTACCTCTTTTGATTGCTCCGGCCT
TCCAGGAGAGATAGAGGACGACCTCTCCCCATA

>ELVd L1

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATCAAACCCCTTACTTTGTAAGT
GTGGTTCGGCGAATGTACCGTTTTCGTCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGT
CGACACCTCTCCCCCTCCCAGGTACTATCCCCTTTCAAGGATGTGTTCCCTAGGAGGGTGGGTGTACCTCTTTTG
GATTGCTCCGGCCTTCCAGGAGAGATAGAGGACGACCTCTCCCCATA

>ELVd L2

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATGGGTCTGGGACTTTAAATTCG
GAGGATTCGTCTTTAAACGTTCCCTCCAAGAGTCCCTTCCCCACGGCGAATGTACCGTTTCGTCTTTTCGGACTC
ATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGTGACACCTCTCCCCCTCCCAGGTACTATCCCCTTTCAA
GGATGTGTTCCCTAGGAGGGTGGGTGTACCTCTTTTGATTGCTCCGGCCTTCCAGGAGAGATAGAGGACGACCT
CTCCCCATA

>ELVd L3

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATTTCGGCGAATGTACCGTTTCG
TCCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGTGACACCTCTCCCCCTCCCAGGTAC
TATCCCCTTTCAAGGATGTGTTCCCTAGGAGGGTGGGTGTACCTCTTTTGATTGCTCCGGCCTTCCAGGAGAGA
TAGAGGACGACCTCTCCCCATA

>ELVd L4

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTGTACCGTTTCGTCTTTTCGGACTCATC
AGGGAAAGTACACACTTTCCGACGGTGGGTTCGTGACACCTCTCCCCCTCCCAGGTACTATCCCCTTTCAAGGA
TGTGTTCCCTAGGAGGGTGGGTGTACCTCTTTTGATTGCTCCGGCCTTCCAGGAGAGATAGAGGACGACCTCTC
CCATA

>ELVd R1

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATGGGTCTGGGACTTTAAATTCG
GAGGATTCGTCTTTAAACGTTCCCTCCAAGAGTCCCTTCCCCAAACCCCTTACTTTGTAAGTGTGGTTCGGCGAAT
GTACCGTTTTCGTCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGTGACACCTCTCCCC
CTCCCAGGTACTATCCCCTTTCAAGGATGTGTTCCCTAGGAGGGTGGGTGTACATAGAGGACGACCTCTCCCCAT
A

>ELVd R2

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATGGGTCTGGGACTTTAAATTCG
GAGGATTCGTCTTTAAACGTTCCCTCCAAGAGTCCCTTCCCCAAACCCCTTACTTTGTAAGTGTGGTTCGGCGAAT
GTACCGTTTTCGTCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGTGACCTCTTTTGGA
TTGCTCCGGCCTTCCAGGAGAGATAGAGGACGACCTCTCCCCATA

>ELVd R3

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATGGGTCTGGGACTTTAAATTCG
GAGGATTCGTCTTTAAACGTTCCCTCCAAGAGTCCCTTCCCCAAACCCCTTACTTTGTAAGTGTGGTTCGGCGAAT
GTACCGTTTTCGTCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGTGACATAGAGGACGAC
CTCTCCCCATA

>ELVd R4

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATGGGTTCGGGACTTTAAATTCG
GAGGATTCGTCTTTAAACGTTTCTCCAAGAGTCCCTTCCCCAACCCCTTACTTTGTAAGTGTGGTTCGGCGAAT
GTACCGTTTCGTCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGCCTCCCCATA

>ELVd L1R3

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATCAAACCCCTTACTTTGTAAGT
GTGGTTCGGCGAATGTACCGTTTCGTCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGT
CATAGAGGACGACCTCTCCCCATA

>ELVd L2R2

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATGGGTTCGGGACTTTAAATTCG
GAGGATTCGTCTTTAAACGTTTCTCCAAGAGTCCCTTCCCCACGGCGAATGTACCGTTTCGTCTTTTCGGACTC
ATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGTGCACCTCTTTTGGATTGCTCCGGCCTTCCAGGAGAGAT
AGAGGACGACCTCTCCCCATA

>ELVd L3R1

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATTGGCGAATGTACCGTTTCG
TCCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGTTCGACACCTTCCCCCTCCAGGTAC
TATCCCCTTTCAAGGATGTGTTCCCTAGGAGGGTGGGTGTACATAGAGGACGACCTCTCCCCATA

>ELVd L3R3

GGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTTCGCCATTGGCGAATGTACCGTTTCG
TCCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCGTGCATAGAGGACGACCTCTCCCCATA

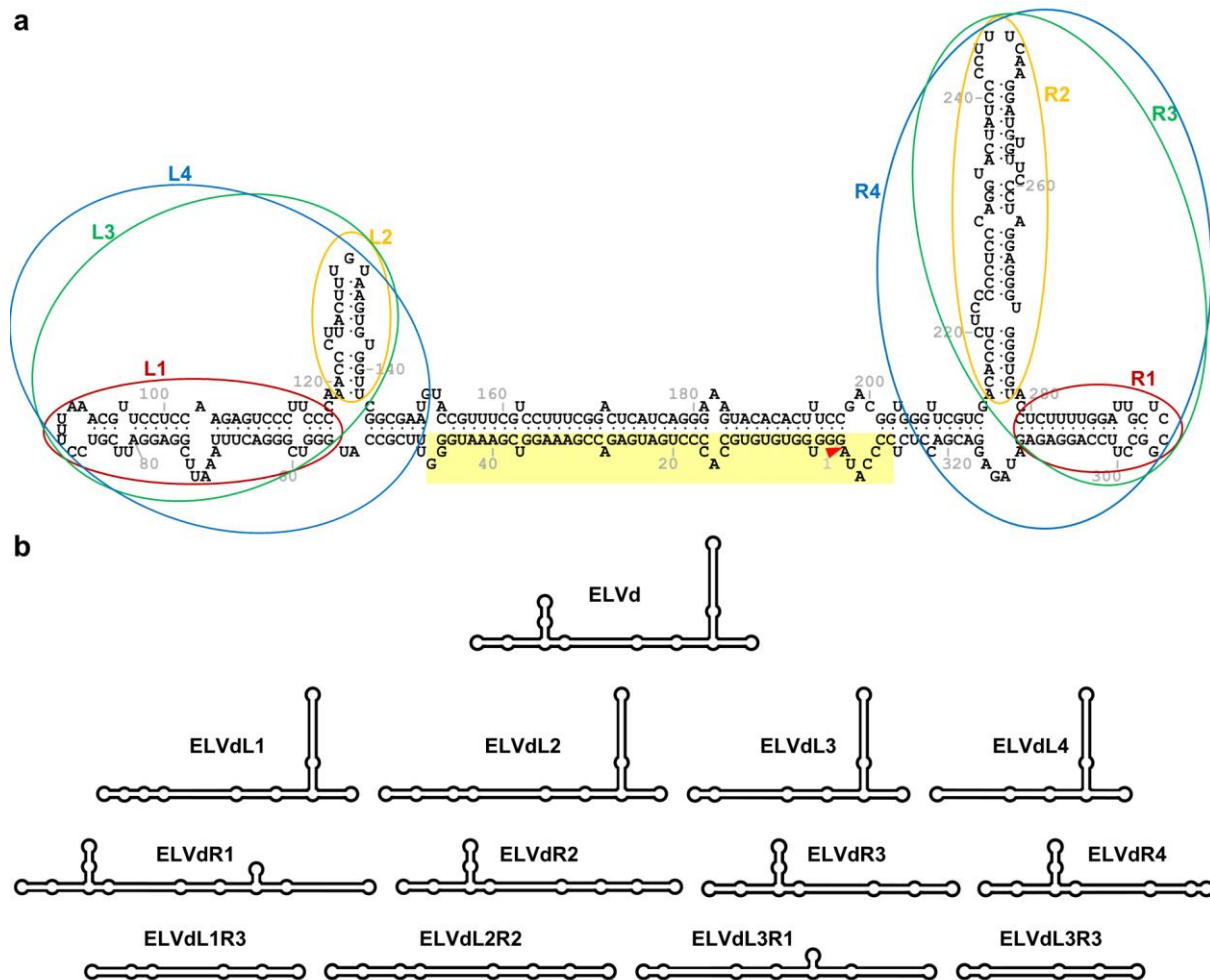


Figure S9. ELVd deleted forms assayed in the viroid-derived system to produce recombinant RNA in *E. coli*. Scheme of the deletions created on the ELVd RNA molecule and assayed in the *E. coli* system to overproduce recombinant RNA. (a) Structural domains of the folded ELVd molecule deleted at the left (L1, L2, L3 and L4) and right (R1, R2, R3 and R4) part of the molecule. (b) Schematic representation of full-length ELVd RNA and the resulting ELVd forms with single deletions at the left (ELVd L1, L2, L3 and L4), at the right (ELVd R1, R2, R3 and R4), and with double deletions at both sides (ELVd L1R3, L2R2, L3R1 and L3R3). RNA secondary structures of minimum free energy were calculated using the Mfold web server (<http://unafold.rna.albany.edu/?q=mfold/RNA-Folding-Form>).

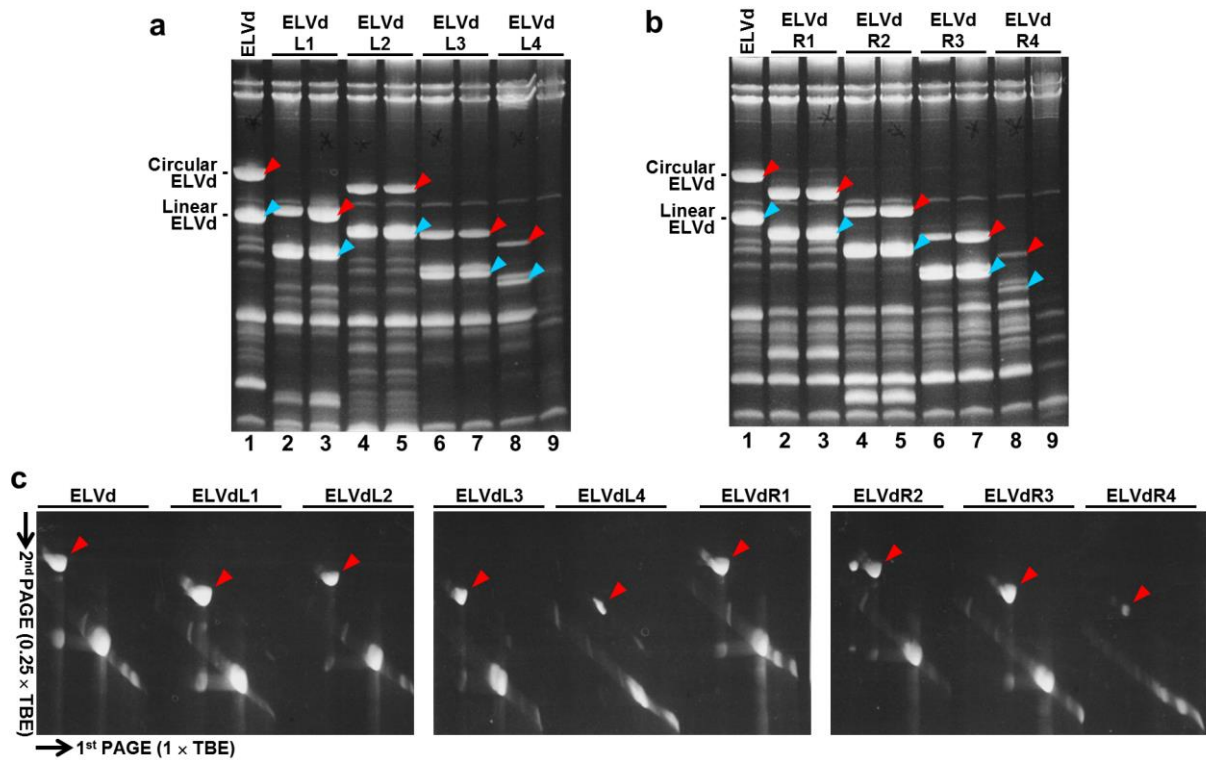


Figure S10. Analysis of the RNA that accumulated in *E. coli* that co-expressed eggplant tRNA ligase and deleted forms of ELVd RNA. Aliquots of the cultures were taken at 8 h post-induction of tRNA ligase expression. Purified RNA was separated by single (**a** and **b**) or double denaturing PAGE (**c**) and stained with ethidium bromide. (**a** and **b**) Lanes 1, RNAs from *E. coli* that expressed full-length ELVd. (**a**) Lanes 2 to 9, RNAs from two independent *E. coli* clones that expressed ELVd forms (see [Supplementary Fig. S9](#)) with deletions at the left side of the molecule, as indicated. (**b**) Lanes 2 to 9, RNAs from two independent *E. coli* clones that expressed ELVd forms with deletions at the right side of the molecule, as indicated. The positions of the circular and linear forms of full-length ELVd RNA are indicated on the left of both panels. The positions of the different circular and linear deleted ELVd RNAs are indicated by red and blue arrowheads, respectively. (**c**) RNAs from *E. coli* that expressed full-length ELVd RNA and ELVd deleted forms L1, L2, L3, L4, R1, R2, R3 and R4, as indicated. The directions of migration of the two electrophoretic separations are indicated. The positions of the corresponding circular RNAs are indicated by a red arrowhead. *E. coli* cultures were grown at 37°C in LB medium and induced at DO₆₀₀ 0.1 with 0.1 mM IPTG. Each lane contains an aliquot of RNA corresponding to 0.8 ml of culture.

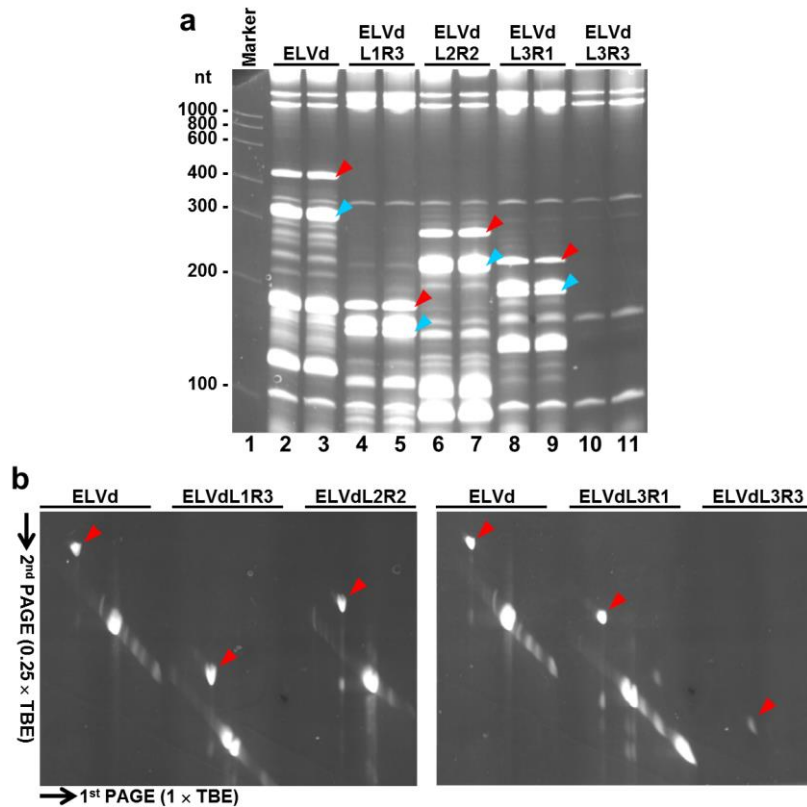


Figure S11. Analysis of the RNA that accumulated in *E. coli* that co-expressed eggplant tRNA ligase and double-deleted ELVd RNA forms. Aliquots of the cultures were taken at 8 h post-induction, RNA purified from the cells and separated by single dimension (a) or double dimension (b) denaturing PAGE. Gels were stained with ethidium bromide. (a) Lane 1, RNA marker with the sizes of the standards indicated on the left in nt; lanes 2 to 11, RNAs from two independent *E. coli* clones that expressed full-length ELVd (lanes 2 and 3) and the ELVd deleted forms L1R3 (lanes 4 and 5), L2R2 (lanes 6 and 7), L3R1 (lanes 8 and 9) and L2R2 (lanes 10 and 11). The positions of the corresponding circular and linear RNAs are indicated by red and blue arrowheads, respectively. (b) RNAs from *E. coli* clones that expressed full-length ELVd and ELVd forms with double deletion (L1R3, L2R2, L3R1 and L3R3), as indicated. The directions of migration of the two electrophoretic separations are indicated. The positions of the corresponding circular RNAs are indicated by a red arrowhead. *E. coli* cultures were grown at 37°C in LB medium and induced at DO₆₀₀ 0.1 with 0.1 mM IPTG. Each lane contains an aliquot of RNA corresponding to 0.8 ml of culture.

Figure S12. Sequence and elements of plasmid p15LtRnlSm used to constitutively express eggplant tRNA ligase in *E. coli*.

```

>p15LtRnlSm (5415 bp)
CGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAAAAATATTTCTCAACATAAAAACTTTG
TGTAACTACTTGTAAACGCTGGGGAGACCACAACGGTTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA
TATACCATGTGCGTTTCGCATAGGGTCATTTACTCTTTCACTCATTACAACTCTATAATCTCTCTTCTTTA
TCATCTTTGCCTTCTAGAATCTTCTTCCCTTTTCAATCTCCTTTCCTTTACACGTTCTCTTCACTCATGCCAAC
AATCAGGAAAGGGGTGGTTATGAAGGAAAAAATGGCAAGTGAGGCCAAGTTCCAATAGGGTACCAGGCTCGTCT
TCAAATGTGGAACCTGTATCTGCTGCAACTGCTGAAGCCATTACCGACCGTCTAAAAGTCCGTGGATATTACTGAA
AGTGGTGCACAGTCTAGTGTTCAGTCACATCTCTTTCAGTTTGGCAGCGTTGGATTAGCACCCCAGTCACCTGTG
CAACATCAAAAAGTAATCTGGAAACCCAAATCATATGGAACAGTGTCTGGAGCCCCAGTGGTTGAAAGCTGGAAAA
ACACCAGTTGAACAAAAAAGTGTCTTTTTAAGTAAATTATTCAAGGGTAAATTTATTGGAAAAATTTACTGTAGAT
AACTCAACATTCTCGAGAGCCCAAGTAAGGGCCACTTTCTACCCAAAATTTGAGAATGAGAAATCAGATCAGGAG
ATCAGGACAAGGATGATAGAGATGGTCTCCAAAGGCTTTGGCTATAGTCGAGGTCACACTTAAGCATTTCTGGATCT
CTTTTTATGTATGCTGGGCATGAAGGTGGAGCATATGCCAAGAATAGCTTCGGGAATATCTATACTGCCGTTGGC
GTCTTTTGTCTTGGACGGATGTTTTCGTGAGGCATGGGGAATAAGCAAGCAAGAAGCAAGCAGATTCATATGAG
TTTTCTTGAGCGCAATCGTATGTGCATATCAATGGAGTTGGTCACGGCAGTGTGGGGGACCAGGACAACGCCCA
CGAGATGATTATGCGGTTGTGACTGCAGTCACGGAGTTGGGAAATGGAAAACCAACTTTCTATTCAACTCCCAGAT
GTAATTGCTTTTTGTCAGGGAATGGCGATTACCAACAAATCATGTATGGCTGTTCTCAACAAGGAAAATCAGTGACT
TCCTTCTTTGCTGCGTATGATGCACCTTTCGAGGAAGGTACAGCAACCACCGTTTTCGAGGCTCTCAGCGAAGTT
GCTGATATTTCTGTACCTGGATCAAAAGACCATATAAAAGTGCAGGGTGAAATTTTGGAGGGTCTCGTGGCCCGC
ATCGTAAAACGTGAGAGCTCAGAGCATATGGAGCGGGTTCTGAGAGATTTTCTCCTCCGCCATCAGAGGGTGAG
GGTTTGGACCTGGGACCTACGTTACGTGAAATTTGTGCTGCAACAGATCAGAAAAGCAGCAAATAAAGGCACTT
CTTCAGAGTGCTGGCACGGCTTTCTGCCCGAATTTATTTGGACTGGTTTGGAGATGAAAACCTCGTTTACATTC
AGAAATGCTGATCGATCTGTTGTCTCAAAGTCTTACAATCACATCCTGCTGATCTTTATACAGGAAAAATACAG
GAAATGGTTTCGCTTGATGAGGGAAAAGCGCTTTTCTGCTGCTTTCAAGTGTCAATTATAACTTACATAAAAATTAAT
GATGTATCGAGTAACAACCTGCCTTTCAAATGGTGATCCATGTATATAGTGATTCAGGCTTCCGCCGGTACCAG
AAAGAGATGAGGCACAAACCAGGACTATGGCCTTTGTATCGAGGCTTTTTTGTGACCTGGATTTATTCAAGGTC
AATGAGAAGAAAACCTGCTGAAATGGCAGGAAGCAACAATCAAATGGTAAAAAATGTGGAAGAGGACAACAGTTTA
GCTGATGAAGATGCAAATCTGATGGTCAAGATGAAATTTCTTACTTACAAGTTGAGAATTTTTTGTATCCGTAAT
GGCTTGTGCACTCTTTTCAAAGAAGGACCTTCTGCGTATAAGTCTTATTACCTGAGGCAAATGAAAATTTGGAAT
ACTTTCAGCAGCCAAGCAACGGAACCTCAGCAAGATGCTTGTATGTAATGGCAGTATATACGCAAGAAAATATGGG
AACAAACCATTGTATCATCATCCACATCACTAAGTGAAGCTGAGCCTTTCTTGAACAATATGCAAAGCCTATGCCA
CAAAATCATGCTTTGATAGGATCTGCTGGAATTTTGTCAAAGTTGAAGATTTTCATGGCTATTTGTGAAGGAGAA
GATGAAGAGGGTGATCTCGAGCCTGCGAAAGATATTGCTCCTTCAAGCCCTAGTATTTCCACCAGAGACATGGTG
GCAAAGAATGAGGGTCTCATTATTTTTCTTTCCAGGAATACCAGGTTGTGCTAAAATCTGCACCTTTGTAAGGAAATA
CTGAATGCTCCAGGAGGGCTTGGAGATGATCGACCAGTTAACAGTTTAAATGGGTGATCTTATTAAAGGTAGATAT
TGGCAAAAAGTTGCTGATGAACGTCGAAGAAAACCTTACTCGATCATGCTTGCTGACAAGATGCACCAAATGAG
GAAGTATGAAAACAAATTTGAGAACATGTGCCTAAGCACCAGGATCTGCTATTCCAGTTATACCTGATTCAGAA
GGAATGAAACTAATCCATTCTCTATTGATGCACCTTTCGGTTTTTATATTCCGAGTACTTCACCGTGTCAATCAT
CCGGGAAATCTTGACAAGTCATCTCAAATGCTGGATATGTGATGCTTATGTTTTATCACCTTATGATGGAAG
AGCCGTCCAGGAGTTCGAGAGTGAGCTTATTGAACGTTTTTGGATCGCTTGTGAGAATTCCTGTACTGAAACCTGAG
AGGTCTCCTCTTCCGATTCTGTGAGGTCTATTATCGAGGAGGGACTCAGTCTGTACAGACTTCATACAACGAAA
CATGGAAGATTGGAGTCTACAAAAGGGACATATGTACAAGAGTGGGTAAAATGGGAGAAAGCAATTGAGAGATATT
CTACTTGGAAATGCAGACTATCTCAATTCAAATACAGGTTCCATTTGAAATTTGCCGTTAAAAGAAAGTCCCTTGAACAA
CTGAAAGTTATTGCGAGGGGGCGAATATGCAGTGCCTGCTGAGAAGAGGAAGCTAGGATCCATTGTATTCGCCGCT
ATCAGCCTGCCAGTTCAGAAATCTAGGTCTTCAAATGATCTAGCAAAGAAAGATCCAAAGGTTGGCGATTTTC
ATTAAGGACAAGAGCATGGAGAGCAGCATTGAGAAGGCCATCTTACCCTGGCTCACAAGAGAAGTACCGGTGTC
ACTGCAGTTGCCAATTACGGTTCTTTCTTTCATCAAAGGTTGCCAGTAGACGTGGCTGCTTTGTTGTTCTCCGAT
AAATTGGCTGCACTAGAAGCTGAGCCTGGCTCTGTTGAAGGTGAAAAGATCAATTTCAAAAACCTCATGCCCCAT
ATCACATTATGGTCTGGTGCAGGAGTTGCCGAAAAGATGCCAATACACTACCACAGTTACTTTCCCAAGGGGAAG
GCTACCCGCATTGATATAAATCCACCGGTCACTATAACTGGCACTCTCGAATTTCTTTCACCACCACCACCACCAC
TGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCCTGAGCAATAACTAGCATAA
CCCCTTGGGGCCTCTAAACGGGTCTTGAAGGGTTTTTTGCTGAAAGGAGATCTGGCGGGGCCGCCTAGCGGAG
TGTATACTGGCTTACTATGTTGGCACTGATGAGGGTGTGAGTGAAGTGTTCATGTGGCAGGAGAAAAAGGCTG
CACCGGTGCGTCAGCAGAATATGTGATACAGGATATATTCCGCTTCTCGCTCACTGACTCGCTACGCTCGGTCCG
TTCGACTGCGGCGAGCGGAAATGGCTTACGAACGGGGCGGAGATTTCTGGAAGATGCCAGGAAGATACTTAAACA
GGGAAGTGAGAGGGCCGCGGCAAGCCGTTTTTCCATAGGCTCCGCCCCCTGACAAGCATCAGGAAATCTGACG
CTCAAATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCTTTCCCCCTGGCGCTCCCTCGTGCC

```


CTCTCCTGTTCCCTGCCTTTTCGGTTTACCGGTGTCATTCCGCTGTTATGGCCGCGTTTGTCTCATTCCACGCCTGA
CACTCAGTTCCGGGTAGGCAGTTTCGCTCCAAGCTGGACTGTATGCACGAACCCCCCGTTTCAGTCCGACCGCTGCC
CCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGAAAGACATGCAAAAAGCACCCTGGCAGCAGCCACTGGTA
ATTGATTTAGAGGAGTTAGTCTTGAAGTCATGCGCCGGTTAAGGCTAAACTGAAAAGGACAAGTTTTGGTGACTGC
GCTCCTCCAAGCCAGTTACCTCGGTTCAAAGAGTTGGTAGCTCAGAGAACCTTCGAAAAACCGCCCTGCAAGGCG
GTTTTTTCGTTTTTCAGAGCAAGAGATTACGCGCAGACCAAAACGATCTCAAGAAGATCATCTTATTAATACGCC
CCGCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAGACGGCA
TGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATATTTGCCCATGGTGAAAACGGGG
GCGAAGAAGTTGTCCATATTGGCCACGTTTAAATCAAACTGGTGAACTCACCCAGGGATTGGCTGAGACGAAA
AACATATTCTCAATAAACCCCTTTAGGGAAATAGGCCAGGTTTTACCGTAACACGCCACATCTTGCGAATATATG
TGTAGAAACTGCCGAAATCGTCGTGGTATTCAGTCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGGAAAACG
GTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCGTCTTTCATTGCCATACGGAATTCCGGATGAGCA
TTCATCAGGCGGGCAAGAATGTGAATAAAGGCCGATAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAG
GCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTA
CGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCTCCATTTTAGCTTCCTTAGCTCCTGAA
AATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTCATTATGGTGAAAGTTGGAAAGCCGGGGCGGC
GGCCGC

E. coli **murein lipoprotein promoter** is in red. Eggplant tRNA ligase (JX0225157) in black with the theoretical amino-terminal **transit peptide** on green background and the carboxy-terminal **hexahistidine tag** in blue. **Stop codon** in red. **T7 bacteriophage terminator** on red background. **p15A replication origin** in gray and **chloramphenicol resistance gene** (reverse orientation) in blue (*promoter* in italics).

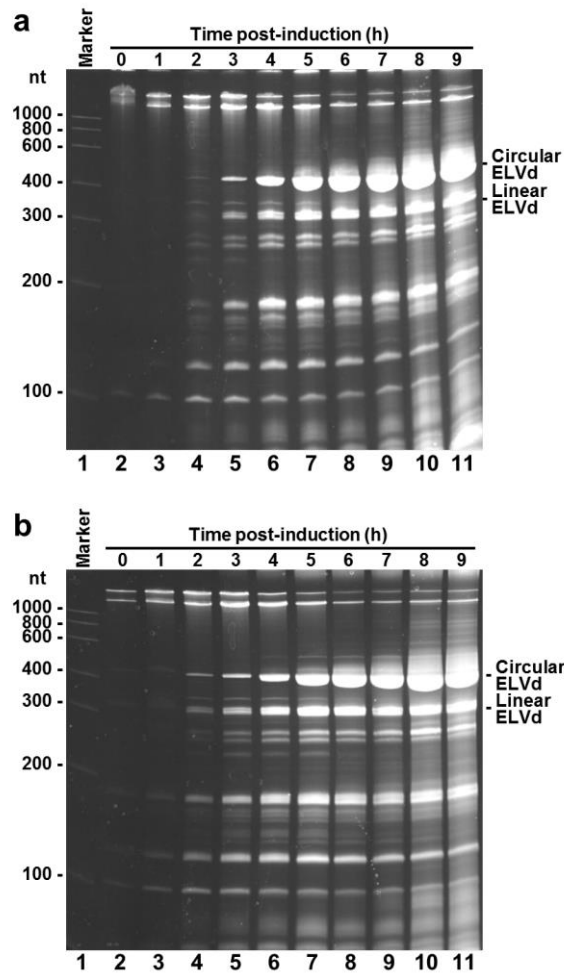


Figure S13. Time-course analysis of the RNA that accumulated in *E. coli* that co-expressed ELVd RNA and eggplant tRNA ligase under the control of (a) IPTG-inducible T7 bacteriophage RNA polymerase and (b) constitutive *E. coli* murein lipoprotein promoters. Aliquots from the cultures were taken at different time points, and RNAs purified and separated by denaturing PAGE. Gels were stained with ethidium bromide. Lanes 1, RNA marker with the sizes of the standards indicated on the left in nt; lanes 2 to 11, RNAs from aliquots taken at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 h post-induction. The positions of the circular and linear ELVd RNAs are indicated on the right. *E. coli* cultures were grown at 37°C in TB medium and induced with 0.1 mM IPTG (a) or not induced (b) at OD₆₀₀ 0.1. Each lane contains an aliquot of RNA corresponding to 0.2 ml of culture.

Figure S14. Sequence and elements of plasmids pLELVd-Spinach and pLELVdL1R3-Spinach.

```

>pLELVd-Spinach (2148 bp)
CGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAAAAATATTCTCAACATAAAAACTTTG
TGTAAACTTTGTAACGCTGCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTT
CGCCATGGGTGCGGACTTTAAATTCGGAGGATTCGTCTTTAAACGTTCCCTCCAAGAGTCCCTTCCCCAACCCCT
TACTTTGTAAGTGTGGTTCGGCGAATGTACCGTTTCGTCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGA
CGGTGGGTTTCGTGACACCTCTCCCCCTCCCAGGTACTATCCCCTTGACGCAACTGAATGAAATGGTGAAGGACG
GGTCCAGGTGTGGCTGCTTCGGCAGTGCAGCTTGTGAGTAGAGTGTGAGCTCCGTAAGTGTGCGCTTCAAGG
ATGTGTTCCCTAGGAGGGTGGGTGTACCTCTTTTGGATTGCTCCGGCTTCCAGGAGAGATAGAGGACGACCTCT
CCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGGAAATCATCCTTAGCGAAAGCT
AAGGATTTTTTTTATCTGAAATGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAA
TCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCT
CGTGCGCTCTCCTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCT
TTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTCGTCCGCTCCAAGCTGGGCTGTGTGCACGAAC
CCCCGTTAGCCCCGACCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAAGACAGCACTTATC
GCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTG
GTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCCGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGAT
TACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGAT
CTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATC
TGGCCCCAGTGTGCAATGATACCGCGAGAGCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGC
CGGAAGGGCCGAGCGCAGAAGTGGTCCGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGC
TAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTC
GTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAA
AAAAGCGGTTAGCTCCTTCGGTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTAT
GGCAGCACTGCATAATTCTTACTGTATGCCATCCGTAAGATGCTTTTTCTGTGACTGGTGAGTACTCAACCAA
GTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCTCAATACGGGATAATACCGCGCCACA
TAGCAGAACTTTAAAGTGTCTATCATTGGAAAACGTTCTTCCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTT
GAGATCCAGTTCGATGTAACCCACTCGTGCACCAACTGATCTTCAGCATCTTTACTTTTACCAGCGTTTCTGG
GTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACT
CTTCCTTTTTCAATATTATTGAAGCATTATCAGGGTATTGTCTCAT

```

E. coli **murein lipoprotein promoter** is in red and **ELVd** cDNA (C327 to G46 of AJ536613) is in black with the repeated hammerhead ribozyme domain on yellow background. cDNA coding for the RNA aptamer **Spinach** in green inserted between T245 and T246 of ELVd cDNA. Ribozymes self-cleavage sites are underlined. *E. coli* ribosomal **rrnC terminator** is in fuchsia. **pUC replication origin** is in gray and **ampicillin resistance gene** (inverse orientation) on gray background (**promoter** on dark gray background).

```

>pLELVdL3R1-Spinach (2030 bp)
CGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAAAAATATTCTCAACATAAAAACTTTG
TGTAAACTTTGTAACGCTGCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTT
CGCCATTCGGCGAATGTACCGTTTCGTCTTTTCGGACTCATCAGGGAAAGTACACACTTTCCGACGGTGGGTTCG
TCGACACCTCTCCCCCTCCCAGGTACTATCCCCTTGACGCAACTGAATGAAATGGTGAAGGACGGGTCCAGGTGT
GGCTGCTTCGGCAGTGCAGCTTGTGAGTAGAGTGTGAGCTCCGTAAGTGTGCGCTTCAAGGATGTGTTCCCT
AGGAGGGTGGGTGTACATAGAGGACGACCTCTCCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGG
TCGAAATGGGGGAAATCATCCTTAGCGAAAGCTAAGGATTTTTTTTATCTGAAATGCGTTGCTGGCGTTTTTCCAT
AGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAA
AGATAACAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCCTGCCGCTTACCGGATACCTG
TCCGCTTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTC
GTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCCAGCCGACCGCTGCGCCTTATCCGGTAACTATCGT
CTTGAGTCCAACCCGGTAAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAGCAGAGCGAGG
TATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATC
TGCCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGT
AGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTTACCAA
TGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTG
TAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGAGCCACGCTCACCG

```

```
GCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCC
TCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCACAACGTTGTT
GCCATTGCTACAGGCATCGTGGTGTACGCTCGTTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCA
AGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGT
AAGTTGGCCGCAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGA
TGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGC
CCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCG
GGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACCTGATCT
TCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATA
AGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTATCAGGGTTATTGT
CTCAT
```

E. coli **murein lipoprotein promoter** is in red and **ELVd L3R1** cDNA (see [Supplementary Dataset 8](#)) is in black with the repeated hammerhead ribozyme domain on yellow background. cDNA coding for the RNA aptamer **Spinach** in green inserted between T245 and T246 of ELVd-AJ536613 cDNA. Ribozymes self-cleavage sites are underlined. *E. coli* ribosomal **rrnC terminator** is in fuchsia. **pUC replication origin** is in gray and **ampicillin resistance gene** (inverse orientation) on gray background (**promoter** on dark gray background).

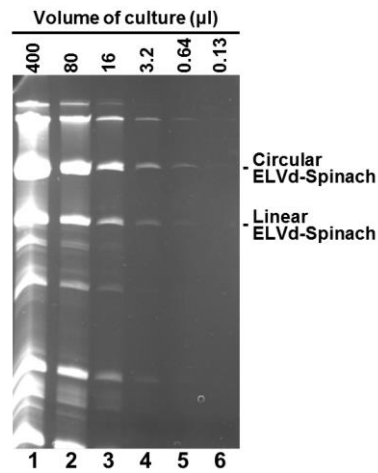


Figure S15. Quantification of the chimeric ELVd-Spinach RNA produced with the viroid-derived system in *E. coli*. The RNA extracted from an aliquot of the culture taken at 12 h post-induction (see Fig. 3a) was subjected to serial dilutions. Samples were separated by denaturing PAGE. The gel was stained with ethidium bromide and the intensities of the bands compared with those in a gel photographed in the same conditions containing known amounts of an RNA standard (*E. coli* 5S rRNA, Supplementary Fig. S7b). Lanes 1 to 6, serial dilutions of the RNA corresponding to 400, 80, 16, 3.2, 0.64 and 0.13 μ l of the original culture, respectively. The positions of the circular and linear ELVd-Spinach RNAs are indicated on the right. *E. coli* cultures were grown at 37°C in TB medium and induced with 0.1 mM ITPG at DO₆₀₀ 0.1.

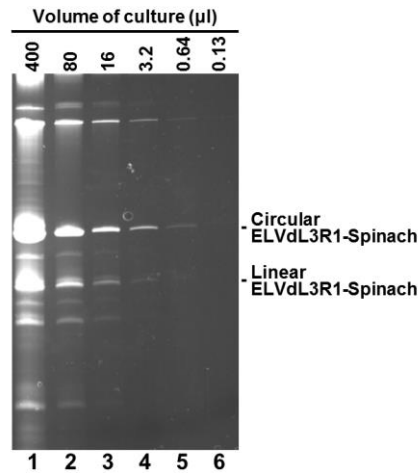


Figure S16. Quantification of the chimeric ELVd L3R1-Spinach RNA produced in *E. coli* using the viroid-derived system. The RNA extracted from the aliquot of the *E. coli* culture taken at 12 h post-induction was subjected to 1/5 serial dilutions and separated by denaturing PAGE. The gel was stained with ethidium bromide and the intensities of the bands compared with those in a gel photographed in the same conditions containing known amounts of an RNA standard (*E. coli* 5S rRNA, [Supplementary Fig. S7b](#)). Lanes 1 to 6, RNAs corresponding to 400, 80, 16, 3.2, 0.64 and 0.13 μ l of the original culture, respectively. The positions of the circular and linear ELVdL3R1-Spinach RNAs are indicated on the right.

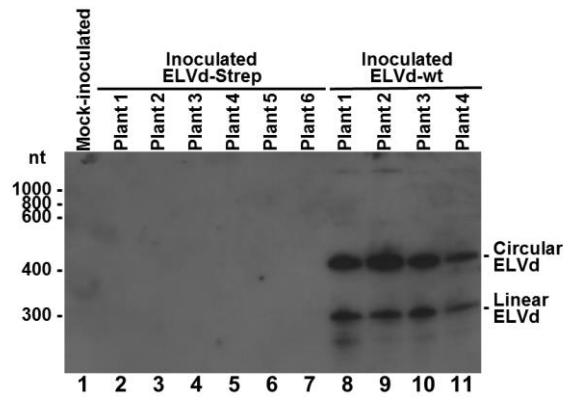


Figure S17. Infectivity of chimeric ELVd-Spinach RNA. Eggplants were mock-inoculated or inoculated with chimeric ELVd-Spinach or wild-type ELVd RNA by means of *Agrobacterium tumefaciens*. Total RNAs were purified from systemic tissue of inoculated plants 45 days post-inoculation, separated by denaturing PAGE, transferred to a membrane and hybridized with an ELVd-specific radioactive RNA probe. The membrane was finally autoradiographed. Lane 1, RNA from a mock-inoculated eggplant; lanes 2 to 7, RNAs from 6 independent eggplants agroinoculated with ELVd-Spinach; lanes 8 to 11, RNAs from 4 independent eggplants agroinoculated with wild-type ELVd. The positions and sizes (in nt) of RNA markers are indicated on the left and the positions of monomeric circular and linear ELVd RNAs on the right of the panel.

Figure S18. Sequences and elements of plasmids pLELVd-hairpin 100, 80, 60 and 40.

>pLELVd-hairpin 100 (2150 bp)

CGATGCTTCTTTGAGCGAACGATCAAAAATAAGTGCCTTCCCATCAAAAAATATTCTCAACATAAAAACTTTG
 TGTAACTACTTGTAAACGCTGCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTT
 CGCCATGGGTCGGGACTTTAAATTCGGAGGATTTCGTCTTTAAACGTTCCCTCCAAGAGTCCCTTCCCCAACCCCT
 TACTTTGTAAAGTGTGGTTCGGCGAATGTACCGTTTCGTCTTTCCGACTCATCAGGGAAAAGTACACACTTTCCGA
 CGGTGGGTTTCGTTCGACACCTCTCCCCCTCCCAGGTAATATCCCTTTCGCAATTTGTTTTTCGTATTTGGATACCTT
 CTTCAAGGTTGGTTATGGGTATACTTCATACCAACCTTGAAGAAGGTATCCAAATACGAAAACAAATTCGCTCAA
 GGATGTGTTCCCTAGGAGGGTGGGTGTACCTCTTTTGGATTGCTCCGGCCTTCCAGGAGAGATAGAGGACGACCT
 CTCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGGAAATCATCCTTAGCGAAAG
 CTAAGGATTTTTTTATCTGAAATGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAA
 AATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCC
 CTCGTGCGCTCTCCTGTTCCGACCTTCCCGCTTACCGGATACCTGTCCGCTTTCCTCCCTTCGGAAGCGTGGCG
 CTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAA
 CCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTA
 TCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAG
 TGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGA
 AAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAG
 ATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCG
 ATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCA
 TCTGGCCCCAGTGTGCAATGATACCGCGAGAGCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCA
 GCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAA
 GCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACCGC
 TCGTCGTTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGC
 AAAAAAGCGGTTAGCTCCTTCGGTCCCTCCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTATCACTCATGGTT
 ATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTTCTGTGACTGGTGAGTACTCAACC
 AAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCTCAATACGGGATAATACCGGCCA
 CATAGCAGAACTTAAAAGTGTCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTG
 TTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTACTTTCACCAGCGTTTCT
 GGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATA
 CTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCAT

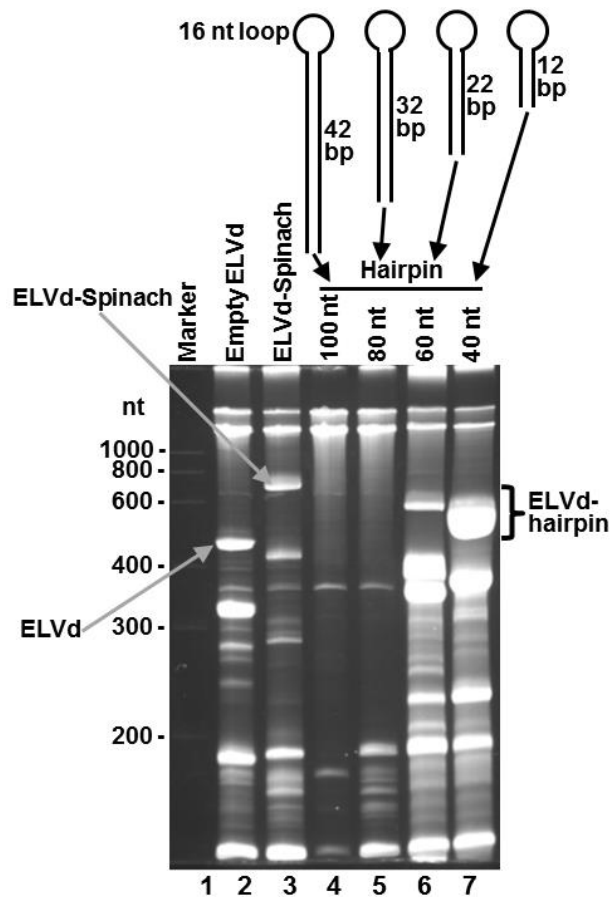
E. coli **murein lipoprotein promoter** is in red and **ELVd** cDNA (C327 to G46 of AJ536613) is in black with the repeated hammerhead ribozyme domain on yellow background. cDNA coding for the **100-nt-long hairpin** structure in blue inserted between T245 and T246 of ELVd cDNA. The sequence corresponding to the **16-nt loop** is underlined. Ribozymes self-cleavage sites are underlined. *E. coli* ribosomal **rrnC terminator** is in fuchsia. **pUC replication origin** is in gray and **ampicillin resistance gene** (inverse orientation) on gray background (**promoter** on dark gray background).

In pLELVd-hairpin 80, 60 and 40, the sequences of the cDNAs coding for the different hairpins of 80, 60 and 40 nt are:

>pLELVd-hairpin 80
 TTTTCGTATTTGGATACCTTCTTCAAGGTTGGTTATGGGTATACTTCATACCAACCTTGAAGAAGGTATCCAAATACGAAA

>pLELVd-hairpin 60
 GGATACCTTCTTCAAGGTTGGTTATGGGTATACTTCATACCAACCTTGAAGAAGGTATCC

>pLELVd-hairpin 40
 TTCAAGGTTGGTTATGGGTATACTTCATACCAACCTTGAA



Supplementary Fig. S19. Production of recombinant RNAs that included extended hairpin structures using the viroid-derived system in *E. coli* BL21(DE3). RNAs were extracted from aliquots of *E. coli* cultures taken at 15:30 h post-inoculation. RNAs were separated by denaturing PAGE and stained with ethidium bromide. Lane 1, RNA marker with the sizes of the standards indicated on the left in nt; lanes 2 to 7, RNAs from *E. coli* transformed to express empty ELVd (lane 2), ELVd-Spinach (lane 3) and different ELVd forms that included hairpin RNAs of 100, 80, 60 and 40 nt as indicated (lanes 4 to 7). All *E. coli* were co-transformed with plasmid p15LtRnlSm. The positions of the circular empty ELVd and ELVd-Spinach are indicated on the left. The positions of some ELVd-hairpin RNAs are indicated on the right. *E. coli* cultures were grown at 37°C in TB medium. Each lane contains an aliquot of RNA corresponding to 0.2 ml of culture.

Figure S20. Sequence and element of plasmid pLELVd-crRNA.

```

>pLELVd-crRNA (2115 bp)
CGATGCTTCTTTGAGCGAACGATCAAAAAATAAGTGCCTTCCCATCAAAAAATATTTCTCAACATAAAAACTTTG
TGTAACTTTGTAACGCTGCCCATAGGGTGGTGTGTGCCACCCCTGATGAGACCGAAAGGTCGAAATGGGGTTT
CGCCATGGGTGGGACTTTAAATTCGGAGGATTTCGTCTTTTAAACGTTTCTCCAAGAGTCCCTTCCCCAAACCTT
TACTTTGTAAGTGTGGTTCGGCGAATGTACCGTTTCGTCTTTTCGGACTCATCAGGGAAAAGTACACACTTTCCGA
CGGTGGGTTCGTGACACCTCTCCCCCTCCCAGGTACTATCCCTTTAATTTCTACTAAGTGTAGATCTGATGGT
CCATGTCTGTTACTCTAATTTCTACTAAGTGTAGATTC AAGGATGTGTTCCCTAGGAGGGTGGGTGTACCTCTTT
TGGATTGCTCCGGCCTTCCAGGAGAGATAGAGGACGACCTCTCCCATAGGGTGGTGTGTGCCACCCCTGATGAG
ACCGAAAGGTCGAAATGGGGGAAATCATCTTAGCGAAAGCTAAGGATTTTTTTTATCTGAAATGCGTTGCTGGC
GTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGAC
AGGATATAAAGATACCAGCGGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCTCTGTTCCGACCTTCCCGCTTAC
CGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTTC
GGTGTAGGTTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGG
TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAG
CAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGT
ATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAAC
CACCGCTGGTAGCGGTGGTTTTTTTTGTTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAAGATCC
TTTTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCTGACT
CCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGAGCC
ACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAAC
TTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGGC
CAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCAGCTCCGGTTC
CCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGT
TGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTTCATGCC
ATCCGTAAGATGCTTTTCTGTGACTGGTGTGACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAG
TTGCTCTTGGCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGTCTCATCTTGGAAA
ACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACC
CAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGAAA
AAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCTTTTTCAATATTTATGAAGCATTTATCA
GGGTTATTGTCTCAT

```

E. coli **murein lipoprotein promoter** is in red and **ELVd** cDNA (C327 to G46 of AJ536613) is in black with the repeated hammerhead ribozyme domain on yellow background. cDNA coding for the **precursor of a Cpf1-based crRNA** in blue inserted between T245 and T246 of ELVd cDNA. Ribozymes self-cleavage sites are underlined. *E. coli* ribosomal **rrnC terminator** is in fuchsia. **pUC replication origin** is in gray and **ampicillin resistance gene** (inverse orientation) on gray background (**promoter** on dark gray background).

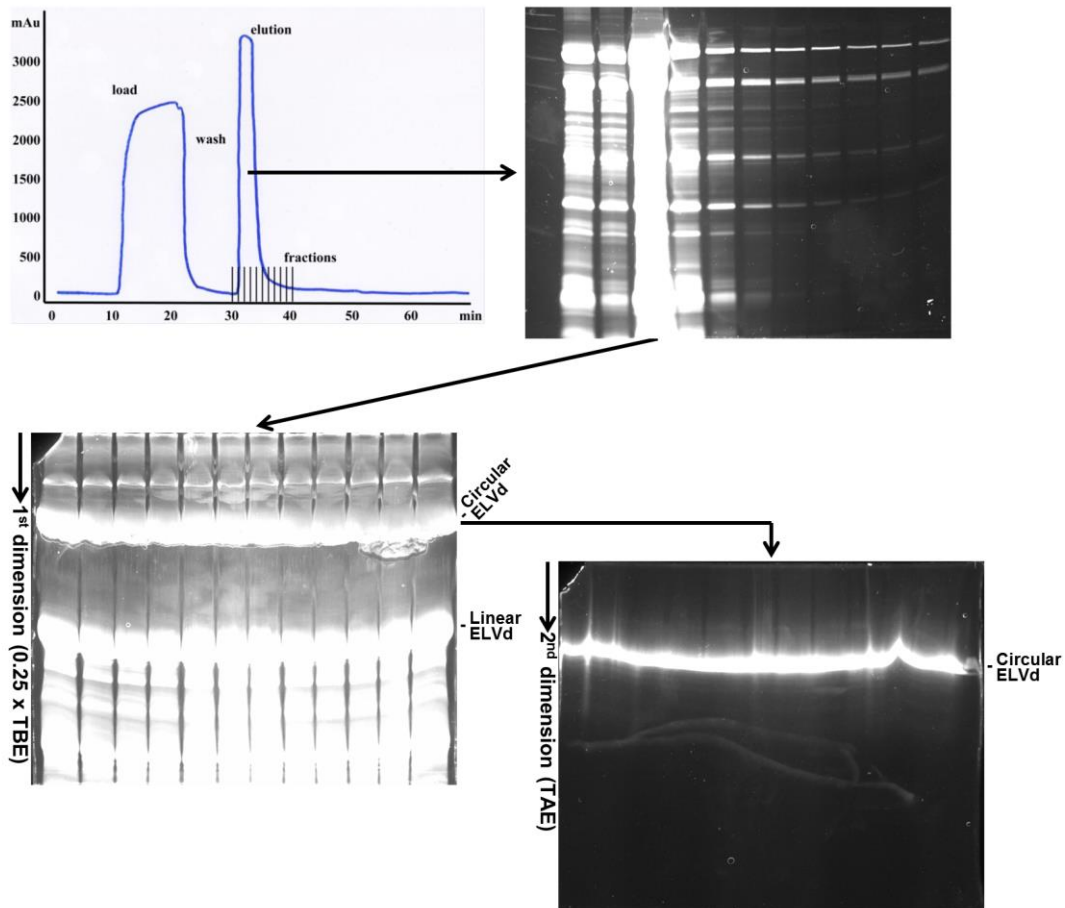


Figure S21. Workflow that illustrates the purification process of a circular RNA produced in *E. coli* using the ELVd-derived system. Total *E. coli* RNAs are first purified by anion exchange chromatography using a DEAE Sepharose column. Aliquots of the different fractions that elute in the presence of 1 M NaCl are analyzed by denaturing (8 M urea) PAGE. Selected fraction is subjected to a two-step electrophoretic separation in polyacrylamide gels. First, RNAs are separated by PAGE in 8 M urea gels in 0.25 x TBE buffer. After ethidium bromide staining of the gel, the band corresponding to the circular RNAs is excised and loaded on top of a second non-denaturing gel that is run in TAE buffer. Circular RNAs are detected in the second gel by ethidium bromide staining and eluted.