

## SUPPORTING INFORMATION

**Table S1.** 10 designed UNS sequences and their properties

| UNS# | Sequence                                   | GC%  | No AT/GC tracts* | Ends with C/G* | No Start Codons* | Restriction sites OK* | Hairpin Max Tm (°C)* | Lacks promoter-like sequences* | Max BLAST Score* |
|------|--|------|------------------|----------------|------------------|-----------------------|----------------------|--------------------------------|------------------|
| 1    | CATTACTCGCATCCATTCTCAGGCTGTCTCGTCTCGTCTC   | 52.5 | ✓                | ✓              | ✓                | ✓                     | 27.1                 | ✓                              | 26.3             |
| 2    | GCTGGGAGTCGTAGACGAAACAAACGAGAACATCCAAGC    | 52.5 | ✓                | ✓              | ✓                | ✓                     | 39.2                 | ✓                              | 32.2             |
| 3    | GCACTGAAGGTCTCAATCGCACTGGAAACATCAAGGTG     | 52.5 | ✓                | ✓              | ✓                | ✓                     | 33.9                 | ✓                              | 26.3             |
| 4    | CTGACCTCCCTGCCAGCAATAGTAAGACAAACACGCAAAGTC | 50.0 | ✓                | ✓              | ✓                | ✓                     | 33.5                 | ✓                              | 28.2             |
| 5    | GAGCCAACCTCCCTTACAACCTCACTCAAGTCGTTAGAG    | 50.0 | ✓                | ✓              | ✓                | ✓                     | 38.5                 | ✓                              | 26.3             |
| 6    | CTCGTTCGCTGCCACCTAAGAATACTCTACGGTCACATAC   | 50.0 | ✓                | ✓              | ✓                | ✓                     | 36.6                 | ✓                              | 26.3             |
| 7    | CAAGACGCTGGCTCTGACATTCCGCTACTGAACACTCG     | 52.5 | ✓                | ✓              | ✓                | ✓                     | 26                   | ✓                              | 26.3             |
| 8    | CCTCGTCTCAACCAAAGCAATCAACCCATCAACCACCTGG   | 52.5 | ✓                | ✓              | ✓                | ✓                     | 39                   | ✓                              | 30.2             |
| X    | CCAGGATACATAGATTACCAACTCCGAGCCCTTCCACC     | 52.5 | ✓                | ✓              | ✓                | ✓                     | 30.7                 | ✓                              | 24.3             |
| 9    | GTTCCCTTATCATGGCGAATCGGACCCACAAGAGCACTG    | 52.5 | ✓                | ✓              | ✓                | ✓                     | 30.8                 | ✓                              | 28.2             |

\* See Methods in main text for specific details of each category.

**Table S2.** Predicted hybridization Tm (°C) of UNS pairs

|   | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | X  | 9  |
|---|----|----|----|----|----|----|----|----|----|----|
| 1 | 81 |    |    |    |    |    |    |    |    |    |
| 2 | -5 | 79 |    |    |    |    |    |    |    |    |
| 3 | -5 | 0  | 79 |    |    |    |    |    |    |    |
| 4 | -5 | -5 | 4  | 79 |    |    |    |    |    |    |
| 5 | -5 | -5 | -5 | -5 | 79 |    |    |    |    |    |
| 6 | -5 | -5 | -5 | -5 | -5 | 78 |    |    |    |    |
| 7 | 5  | -5 | 1  | -5 | -5 | 7  | 80 |    |    |    |
| 8 | -5 | -5 | -2 | 3  | 6  | -5 | -5 | 79 |    |    |
| X | -5 | -5 | -5 | -5 | -5 | -5 | -5 | 16 | 78 |    |
| 9 | -5 | -5 | 18 | -5 | -5 | -5 | -2 | 18 | 17 | 81 |

**Table S3. Part and destination vectors used in this work**

| Basic Part vectors        |   |   |            |                                       |                 |                   |                 |
|---------------------------|---|---|------------|---------------------------------------|-----------------|-------------------|-----------------|
| Plasmid                   | Part Contains:  | Construction  | Resistance | Copy Number                           | UN Digest Sites | UN+1 Digest Sites | UX Digest Sites |
| pFL_U1U2                  | U1-MCS-U2-UX  | MfeI/NsiI digested gBlock_U1U2_FL was ligated into EcoRI/PstI digested BioBrick-modified pIDTSmart vector (IDT).  | Amp        | ~500                                  | Ascl/BspMI      | MauBI/BbsI        | Mrel/Bsal       |
| pFL_U2U3                  | U2-MCS-U3-UX  | MfeI/NsiI digested gBlock_U2U3_FL was ligated into EcoRI/PstI digested BioBrick-modified pIDTSmart vector (IDT).  | Amp        | ~500                                  | Ascl/BspMI      | MauBI/BbsI        | Mrel/Bsal       |
| pFL_U3U4                  | U3-MCS-U4-UX  | MfeI/NsiI digested gBlock_U3U4_FL was ligated into EcoRI/PstI digested BioBrick-modified pIDTSmart vector (IDT).  | Amp        | ~500                                  | Ascl/BspMI      | MauBI/BbsI        | Mrel/Bsal       |
| pFL_U4U5                  | U4-MCS-U5-UX  | MfeI/NsiI digested gBlock_U4U5_FL was ligated into EcoRI/PstI digested BioBrick-modified pIDTSmart vector (IDT).  | Amp        | ~500                                  | Ascl/BspMI      | MauBI/BbsI        | Mrel/Bsal       |
| pJT170                    | U1-PT7-MCS-TT7-U2-UX  | pETDuet-1 digested with XbaI, blunted with klenow fragment, and digested again with DralII, followed by ligation with phosphorylated, DralII-digested gBlockU1U2. SphI/MfeI then digested, blunted, and re-ligated. | Amp        | ~40                                   | AfIII/SapI      | MauBI/BbsI        | Mrel/Bsal       |
| pJT172                    | U2-PT7-MCS-TT7-U3-UX  | pETDuet-1 digested with XbaI, blunted with klenow fragment, and digested again with DralII, followed by ligation with phosphorylated, DralII-digested gBlockU2U3. SphI/MfeI then digested, blunted, and re-ligated. | Amp        | ~40                                   | AfIII/SapI      | MauBI/BbsI        | Mrel/Bsal       |
| pJT174                    | U3-PT7-MCS-TT7-U4-UX  | pETDuet-1 digested with XbaI, blunted with klenow fragment, and digested again with DralII, followed by ligation with phosphorylated, DralII-digested gBlockU3U4. SphI/MfeI then digested, blunted, and re-ligated. | Amp        | ~40                                   | AfIII/SapI      | MauBI/BbsI        | Mrel/Bsal       |
| pJT176                    | U4-PT7-MCS-TT7-U5-UX  | pETDuet-1 digested with XbaI, blunted with klenow fragment, and digested again with DralII, followed by ligation with phosphorylated, DralII-digested gBlockU4U5. SphI/MfeI then digested, blunted, and re-ligated. | Amp        | ~40                                   | AfIII/SapI      | MauBI/BbsI        | Mrel/Bsal       |
| pJT260                    | U1-Ptrc-mCherry-[TB1006]^2-TT7-U2-UX  | Described below.  | Amp        | ~40                                   | AfIII/SapI      | MauBI/BbsI        | Mrel/Bsal       |
| pJT288                    | U2-Ptrc-mCherry-[TB1006]^2-TT7-U3-UX  | ClaI/Xhol fragment of pJT260 cloned into ClaI/Xhol-digested pJT172.   | Amp        | ~40                                   | AfIII/SapI      | MauBI/BbsI        | Mrel/Bsal       |
| pJT290                    | U3-Ptrc-mCherry-[TB1006]^2-TT7-U4-UX  | ClaI/Xhol fragment of pJT260 cloned into ClaI/Xhol-digested pJT174.   | Amp        | ~40                                   | AfIII/SapI      | MauBI/BbsI        | Mrel/Bsal       |
| pJT292                    | U4-Ptrc-mCherry-[TB1006]^2-TT7-U5-UX  | ClaI/Xhol fragment of pJT260 cloned into ClaI/Xhol-digested pJT176.   | Amp        | ~40                                   | AfIII/SapI      | MauBI/BbsI        | Mrel/Bsal       |
| Basic Destination vectors |   |   |            |                                       |                 |                   |                 |
| Plasmid                   | Destination UNSes & Backbone:   | Construction  | Resistance | Copy Number                           | U1 Digest Sites | UX Digest Sites   |                 |
| pDestET                   | U1-UX. Backbone:<br>pETDuet-1 (Novagen)<br>lacking promoter, MCS,<br>T7Term and F1 Ori. | gBlock_U1UX_A digested with SphI/XbaI and ligated into SphI/AvrII-digested pETDuet-1. Resulting plasmid then digested with BlpI/SspI to eliminate F1 Ori, blunted with Klenow fragment, and re-ligated.             | Amp        | ~40                                   | MauBI/BbsI      | AvrII/Bpml        |                 |
| pDestBAC                  | U1-UX. Backbone:<br>pETCoCo-1 (Novagen)<br>with stop codon at NheI site.                | pETCoCo-1 digested with NheI, blunted with KF, and re-ligated to generate a stop codon. Resulting plasmid then digested with HindIII/AvrII and HindIII/XbaI digested gBlock_U1UX_B ligated in.                      | Cam        | ~1 (Amplifiable to 40 with arabinose) | MauBI/BbsI      | AvrII/Bpml        |                 |

|               |  |  |     |                                       |            |            |  |
|---------------|--|--|-----|---------------------------------------|------------|------------|--|
| pDestRmce BAC | U1-UX. Backbone: pETCoco-1 containing cassette for RMCE.                   | Multiple steps. In short, the T7 Promoter, MCS and LoxP site from pETCoco-1 were removed and replaced with a U1-UX cassette and the inverted LoxP sites required for RMCE-based integration (Ref. #33 in main text).   | Cam | ~1 (Amplifiable to 40 with arabinose) | MauBI/BbsI | AvrII/BpmI |  |
| pDestPB BAC   | U1-UX. Backbone: pETCoco-1 containing cassette for PiggyBAC transposition. | Multiple steps. In short, the T7 Promoter and MCS from pETCoco-1 were removed and replaced with inverted terminal repeats (ITRs) containing the U1-UX cassette, HS4 and a blasticidin resistance gene. The PiggyBAC transposase was cloned outside of the ITRs. (Ref. #33 in main text). | Cam | ~1 (Amplifiable to 40 with arabinose) | MauBI/BbsI | AvrII/BpmI |  |

#### Part vectors for terminator testing

| Plasmid | Part Contains:                       | Construction   | Resistance | Copy Number | UN Digest Site | UN+1 Digest Site | UX Digest Site |
|---------|--------------------------------------|--|------------|-------------|----------------|------------------|----------------|
| pJT233  | U1-PT7-mCherry-TT7-U2-UX             | Ncol/XbaI mCherry fragment of pDHL374 ligated into Ncol/Spel-digested pJT170.  | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT246  | U1-Ptrc-mCherry-TT7-U2-UX            | Ptrc fragments were generated by annealing primers JT326 and JT328, filling in the gaps with Klenow fragment, digesting with Clal/XbaI, and ligating into Clal/XbaI-digested pJT233. | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT257  | U1-Ptrc-mCherry-TB1006-TT7-U2-UX     | BBa_B1006 terminator fragments were generated by annealing phosphorylated primers JT356 and JT357, and ligating into Nhel/PstI-digested pJT246.                                      | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT260  | U1-Ptrc-mCherry-[TB1006]^2-TT7-U2-UX | BBa_B1006 terminator fragment ligated into Spel/PstI-digested pJT257.  | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT321  | U1-Ptrc-mCherry-[TB1006]^3-TT7-U2-UX | BBa_B1006 terminator fragment ligated into Spel/PstI-digested pJT260.  | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT318  | U1-Ptrc-mCherry-U2-UX                | pJT260 digested with BamHI/Agel, blunted with klenow fragment, and re-ligated to remove all terminators.   | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT320  | U1-Ptrc-mCherry-TT7-U2-UX            | pJT260 digested with BamHI/Spel, blunted with klenow fragment, and re-ligated to remove all but the T7term.  | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT241  | U2-PT7-EGFP-TT7-U3-UX                | Ncol/XbaI EGFP fragment from pEGFP (Clontech) ligated into Ncol/Spel-digested pJT172.  | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT323  | U1-Ptrc-EGFP-[TB1006]^2-TT7-U2-UX    | Ncol/BamHI EGFP fragment of pJT241 ligated into Ncol/BamHI-digested pJT260.  | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT336  | U2-Ptrc-EGFP-[TB1006]^2-TT7-U3-UX    | XbaI/Xhol fragment of pJT323 ligated into XbaI/Xhol-digested pJT288.   | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT345  | U2-EGFP-[TB1006]^2-TT7-U3-UX         | pJT336 digested with Clal/XbaI, blunted with klenow fragment, and re-ligated to remove the promoter.   | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |

#### Part vectors for testing UNS effects on proximal expression cassettes

| Plasmid | Part Contains:                  | Construction   | Resistance | Copy Number | UN Digest Site | UN+1 Digest Site | UX Digest Site |
|---------|---------------------------------|--|------------|-------------|----------------|------------------|----------------|
| pJT316  | U1-mCherry-[TB1006]^2-TT7-U2-UX | pJT260 digested with Clal/XbaI, blunted with klenow fragment, and re-ligated to remove the promoter. | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT339  | U2-mCherry-[TB1006]^2-TT7-U3-UX | pJT288 digested with Clal/XbaI, blunted with klenow fragment, and re-ligated to remove the promoter. | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |
| pJT341  | U3-mCherry-[TB1006]^2-TT7-U4-UX | pJT290 digested with Clal/XbaI, blunted with klenow fragment, and re-ligated to remove the promoter. | Amp        | ~40         | AflII/SapI     | MauBI/BbsI       | Mrel/Bsal      |

|        |                                      |  |     |     |            |            |           |
|--------|--------------------------------------|--|-----|-----|------------|------------|-----------|
| pJT343 | U4-mCherry-[TB1006]^2-TT7-U5-UX      | pJT292 digested with Clal/XbaI, blunted with klenow fragment, and re-ligated to remove the promoter. | Amp | ~40 | AfIII/Sapl | MauBI/BbsI | Mrel/Bsal |
| pJT260 | U1-Ptrc-mCherry-[TB1006]^2-TT7-U2-UX | Described above  | Amp | ~40 | AfIII/Sapl | MauBI/BbsI | Mrel/Bsal |
| pJT288 | U2-Ptrc-mCherry-[TB1006]^2-TT7-U3-UX | Described above  | Amp | ~40 | AfIII/Sapl | MauBI/BbsI | Mrel/Bsal |
| pJT290 | U3-Ptrc-mCherry-[TB1006]^2-TT7-U4-UX | Described above  | Amp | ~40 | AfIII/Sapl | MauBI/BbsI | Mrel/Bsal |
| pJT292 | U4-Ptrc-mCherry-[TB1006]^2-TT7-U5-UX | Described above  | Amp | ~40 | AfIII/Sapl | MauBI/BbsI | Mrel/Bsal |

#### Part vectors for promoter titration and mCherry/EGFP library construction

| Plasmid | Part Contains:                       | Construction   | Resistance | Copy Number | UN Digest Site | UN+1 Digest Site | UX Digest Site |
|---------|--------------------------------------|--|------------|-------------|----------------|------------------|----------------|
| pJT301  | U1-P740-mCherry-[TB1006]^2-TT7-U2-UX | Primers JT407/408 phosphorylated, annealed and ligated into Clal/XbaI-digested pJT260. | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |
| pJT303  | U1-P199-mCherry-[TB1006]^2-TT7-U2-UX | Primers JT409/410 phosphorylated, annealed and ligated into Clal/XbaI-digested pJT260. | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |
| pJT305  | U1-P048-mCherry-[TB1006]^2-TT7-U2-UX | Primers JT411/412 phosphorylated, annealed and ligated into Clal/XbaI-digested pJT260. | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |
| pJT309  | U1-P003-mCherry-[TB1006]^2-TT7-U2-UX | Primers JT415/416 phosphorylated, annealed and ligated into Clal/XbaI-digested pJT260. | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |
| pJT353  | U2-P740-EGFP-[TB1006]^2-TT7-U3-UX    | Primers JT407/408 phosphorylated, annealed and ligated into Clal/XbaI-digested pJT336. | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |
| pJT351  | U2-P199-EGFP-[TB1006]^2-TT7-U3-UX    | Primers JT409/410 phosphorylated, annealed and ligated into Clal/XbaI-digested pJT336. | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |
| pJT349  | U2-P048-EGFP-[TB1006]^2-TT7-U3-UX    | Primers JT411/412 phosphorylated, annealed and ligated into Clal/XbaI-digested pJT336. | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |
| pJT347  | U2-P003-EGFP-[TB1006]^2-TT7-U3-UX    | Primers JT415/416 phosphorylated, annealed and ligated into Clal/XbaI-digested pJT336. | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |

#### Deoxychromoviridans part vectors

| Plasmid | Part Contains:                    | Construction   | Resistance | Copy Number | UN Digest Site | UN+1 Digest Site | UX Digest Site |
|---------|-----------------------------------|--|------------|-------------|----------------|------------------|----------------|
| pJT369  | U1-P003-VioB-[TB1006]^2-TT7-U2-UX | Primers JT446/447 phosphorylated, annealed and ligated into pVioB to remove internal XbaI site. XbaI-Sspl fragment of resulting vector then cloned into XbaI/StuI-digested pJT309. | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |
| pJT375  | U2-P003-VioA-[TB1006]^2-TT7-U3-UX | XbaI-BsaAI fragment of pVioA ligated into BamHI-BLUNT/XbaI digested pJT347.  | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |
| pJT371  | U3-Ptrc-VioE-[TB1006]^2-TT7-U4-UX | XbaI-BsaAI fragment of pVioE ligated into XbaI/EcoRV-digested pJT290.  | Amp        | ~40         | AfIII/Sapl     | MauBI/BbsI       | Mrel/Bsal      |

#### AND logic gate part vectors

| Plasmid | Part Contains:    | Details  | Resistance | Copy Number | UN Digest Site | UN+1 Digest Site | UX Digest Site |
|---------|-------------------|--|------------|-------------|----------------|------------------|----------------|
| pFL_A   | U1_HS4_U2         | HS4 (derived from constructs in Lienert et al. 2013, Ref. #33 in main text). | Amp        | ~500        | Ascl/BspMI     | MauBI/BbsI       | Mrel/Bsal      |
| pFL_B   | U2_HSVreporter_U3 | 6xTAL118bs-spacer_TK_2xCFP_NLS_STOP_BGHterm_HS4 (derived from                | Amp        | ~500        | Ascl/BspMI     | MauBI/BbsI       | Mrel/Bsal      |

|        |                    |  |     |      |            |            |           |
|--------|--------------------|--|-----|------|------------|------------|-----------|
|        |                    | constructs in Lienert et al. 2013, Ref. #33 in main text).   |     |      |            |            |           |
| pFL_C1 | U3_C-input_U4      | CMV-intC-TAL118-a-C2_t2A_mCherry_BGHterm_HS4 (derived from constructs in Lienert et al. 2013, Ref. #33 in main text).        | Amp | ~500 | Ascl/BspMI | MauBI/BbsI | Mrel/Bsal |
| pFL_C2 | U3_C-input_A_U4    | CMV-intC-TAL118-a-C2_BGHterm_HS4 (derived from constructs in Lienert et al. 2013, Ref. #33 in main text).                    | Amp | ~500 | Ascl/BspMI | MauBI/BbsI | Mrel/Bsal |
| pFL_D1 | U4_N-input_U5      | CMV-TAL118-a-N2-intN_NLS_BGHterm_HS4 (derived from constructs in Lienert et al. 2013, Ref. #33 in main text).                | Amp | ~500 | Ascl/BspMI | MauBI/BbsI | Mrel/Bsal |
| pFL_D2 | U4_mutN-input_U5   | CMV-mutTAL118-a-N2-intN_NLS_BGHterm_HS4 (derived from constructs in Lienert et al. 2013, Ref. #33 in main text).             | Amp | ~500 | Ascl/BspMI | MauBI/BbsI | Mrel/Bsal |
| pFL_D3 | U4_N-input_A_U5    | CMV-TAL118-a-N2-intN_NLS_t2A_mCherry_BGHterm_HS4 (derived from constructs in Lienert et al. 2013, Ref. #33 in main text).    | Amp | ~500 | Ascl/BspMI | MauBI/BbsI | Mrel/Bsal |
| pFL_D4 | U4_mutN-input_A_U5 | CMV-mutTAL118-a-N2-intN_NLS_t2A_mCherry_BGHterm_HS4 (derived from constructs in Lienert et al. 2013, Ref. #33 in main text). | Amp | ~500 | Ascl/BspMI | MauBI/BbsI | Mrel/Bsal |

**Plasmids obtained from companies or other labs**

| Plasmid   | Contains:              | From:                           | Resistance | Copy Number                           | UN Digest Site | UN+1 Digest Site | UX Digest Site |
|-----------|------------------------|---------------------------------|------------|---------------------------------------|----------------|------------------|----------------|
| pDHL374   | mCherry                | Per Malkus, Johan Paulsson Lab  | Amp        | ~1-5                                  | NA             | NA               | NA             |
| pEGFP     | EGFP                   | Clontech                        | Amp        | ~500                                  | NA             | NA               | NA             |
| pVioA     | VioA-His6 in pET24b    | Tim Wencewicz, Chris Walsh Lab. | Kan        | ~40                                   | NA             | NA               | NA             |
| pVioB     | VioB-His6 in pET24b    | Tim Wencewicz, Chris Walsh Lab. | Kan        | ~40                                   | NA             | NA               | NA             |
| pVioE     | VioE-His6 in pET24b    | Tim Wencewicz, Chris Walsh Lab. | Kan        | ~40                                   | NA             | NA               | NA             |
| pETDuet-1 | T7 expression cassette | Novagen                         | Amp        | ~40                                   | NA             | NA               | NA             |
| pETCoco-1 | T7 expression cassette | Novagen                         | Cam        | ~1 (Amplifiable to 40 with arabinose) | NA             | NA               | NA             |

**Table S4. Primers used in this study**

| Designation | Name              | Length | Sequence   |
|-------------|-------------------|--------|--|
| JT326       | Plac.Rev          | 52     | GAT CTC TAG AGG GGA ATT GTT ATC CGC TCA CAA TTC CAC ACA TTA TAC GAG C                          |
| JT328       | Plac.Fwd.19bpTRC  | 54     | GAC TAT CGA TGC TGT TGA CAA TTA ATC ATC CGG CTC GTA TAA TGT GTG GAA TTG                        |
| JT356       | BBa_B1006_TOP     | 63     | /5Phos/CTA GCA AAA AAA AAC CCC GCC CCT GAC AGG GCG GGG TTT TTT TTA CTA GTG CGG CCG CCT GCA     |
| JT357       | BBa_B1006_BOT     | 55     | /5Phos/GGC GGC CGC CGC ACT AGT AAA AAA AAC CCC GCC CTG TCA GGG GCG GGG TTT TTT G               |
| JT407       | ClalXbal_740_Top  | 69     | CGA TGC TGT TGA CAA TTA ATC ATC CGG CTC ATA AAA TTT GTG GAA TTG TGA GCG GAT AAC AAT TCC CCT    |
| JT408       | ClalXbal_740_Bot  | 71     | CTA GAG GGG AAT TGT TAT CCG CTC ACA ATT CCA CAA ATT TTA TGA GCC GGA TGA TTA ATT GTC AAC AGC AT |
| JT409       | ClalXbal_199_Top  | 69     | CGA TGC TGT TGA CAA TTA ATC ATC CGG CTC GTA GTG TCT GTG GAA TTG TGA GCG GAT AAC AAT TCC CCT    |
| JT410       | ClalXbal_199_Bot  | 71     | CTA GAG GGG AAT TGT TAT CCG CTC ACA ATT CCA CAG ACA CTA CGA GCC GGA TGA TTA ATT GTC AAC AGC AT |
| JT411       | ClalXbal_48_Top   | 69     | CGA TGC TGT TAC AAC TTA ATC ATC CGG CTC GTA TAA TGT GTG GAA TTG TGA GCG GAT AAC AAT TCC CCT    |
| JT412       | ClalXbal_48_Bot   | 71     | CTA GAG GGG AAT TGT TAT CCG CTC ACA ATT CCA CAC ATT ATA CGA GCC GGA TGA TTA AGT TGT AAC AGC AT |
| JT413       | ClalXbal_12_Top   | 69     | CGA TGC TGT TGG GGC TTA ATC ATC CGG CTC GTA TAA TGT GTG GAA TTG TGA GCG GAT AAC AAT TCC CCT    |
| JT414       | ClalXbal_12_Bot   | 71     | CTA GAG GGG AAT TGT TAT CCG CTC ACA ATT CCA CAC ATT ATA CGA GCC GGA TGA TTA AGC CCC AAC AGC AT |
| JT415       | ClalXbal_3_Top    | 69     | CGA TGC TGT TTT TAA TTA ATC ATC CGG CTC GTA TTG TAT GTG GAA TTG TGA GCG GAT AAC AAT TCC CCT    |
| JT416       | ClalXbal_3_Bot    | 71     | CTA GAG GGG AAT TGT TAT CCG CTC ACA ATT CCA CAT ACA ATA CGA GCC GGA TGA TTA ATT AAA AAC AGC AT |
| JT417       | ClalXbal_0.75_Top | 69     | CGA TGC TGT TTT GGT TTA ATC ATC CGG CTC CTA CTC TGT GTG GAA TTG TGA GCG GAT AAC AAT TCC CCT    |
| JT418       | ClalXbal_0.75_Bot | 71     | CTA GAG GGG AAT TGT TAT CCG CTC ACA ATT CCA CAC AGA GTA GGA GCC GGA TGA TTA AAC CAA AAC AGC AT |
| JT446       | VioB_RE_Fix_TOP   | 45     | /5Phos/AGC TCT CCA GAG AGG CCC TTG AGC ACC ACC ACC ACC ACT GAC                                 |
| JT447       | VioB_RE_Fix_BOT   | 45     | /5Phos/TCG AGT CAG TGG TGG TGG TGG TGC TCA AGG GCC TCT CTG GAG                                 |

**Table S5. gBlocks used in this study**

| gBlock Name    | Sequence  |
|----------------|---|
| gBlock_U1UX_A  | GACTGCATGCGCTTCTTAAGCATTACTCGCATCCATTCTCAGGCTGTCGTCGTCGCTCCGCCGGTCTGGAGATGATCAAACCTAGGCCAGGATA<br>CATAGATTACCACAACCTCCGAGCCCTCCACCCGCCGGAGACCTAGAGATC   |
| gBlock_U1UX_B  | GATCAGATCTGTGAGCGGCCGATGAAAGCTGCTTCTTAAGCATTACTCGCATCCATTCTCAGGCTGTCGTCGTCGCTCCGCCGGTCTGGAG<br>ATGATCAAACCTAGGCCAGGATACTAGATTACCACAACCTCCGAGCCCTCCACCCGCCGGAGACCTAGAGATC  |
| gBlock_U1U2    | GACTCAATTGCTTCTTAAGCATTACTCGCATCCATTCTCAGGCTGTCGTCGTCGCTCCGCCGGTCTGGAGATGATCTCGATCCCGCGAAATTAAATACGACT<br>CACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAAATAATTGGTTAACCTTAAGAAGGAGATATAACCATGGGAGATCTACTAGTAGCAGCCATCA<br>CCATCATCACCACAGCGGATCCGCTAGCTGACTGCAGAGAAGCTGGCCGCCCTCGAGTCGCTAGGCCGCTGAGCAATAACTAGCATAACCCCTGGGCC<br>TCTAAACGGGTCTTGAGGGGTTTTGCTGAAAACCTCAGGACCGGGTGGACTGAAGGTCTCAATCGCACTGGAAACATCAAGGTCGCGCGCGGTCTCG<br>CCAGGATACATAGATTACCACAACCTCGAGCCCTCCACCCGCCGGAGACCCACGTAGTGAGTC               |
| gBlock_U2U3    | GACTCAATTGCTTCTTAAGGCTGGAGTTCGTAGACGGAAACAAACGCAGAATCCAAGGCCCTGCATTAGGATGATCTCGATCCCGCGAAATTAAATACGAC<br>TCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAAATAATTGGTTAACCTTAAGAAGGAGATATAACCATGGGAGATCTACTAGTAGCAGCCATC<br>ACCATCATCACCACAGCGGATCCGCTAGCTGACTGCAGAGAAGCTGGCCGCCCTCGAGTCGCTAGGCCGCTGAGCAATAACTAGCATAACCCCTGGGCC<br>CTCTAAACGGGTCTTGAGGGGTTTTGCTGAAAACCTCAGGACCGGGTGGACTGAAGGTCTCAATCGCACTGGAAACATCAAGGTCGCGCGCGGTCTCG<br>GCCAGGATACATAGATTACCACAACCTCGAGCCCTCCACCCGCCGGAGACCCACGTAGTGAGTC             |
| gBlock_U3U4    | GACTCAATTGCTTCTTAAGGCTCTCAATCGCAGTAAGGCTGGAGTTCGTAGACGGAAACATCAAGGTCGCGCCCTGCATTAGGATGATCTCGATCCCGCGAAATTAAATACGAC<br>TCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAAATAATTGGTTAACCTTAAGAAGGAGATATAACCATGGGAGATCTACTAGTAGCAGCCATC<br>ACCATCATCACCACAGCGGATCCGCTAGCTGACTGCAGAGAAGCTGGCCGCCCTCGAGTCGCTAGGCCGCTGAGCAATAACTAGCATAACCCCTGGGCC<br>CTCTAAACGGGTCTTGAGGGGTTTTGCTGAAAACCTCAGGACCGGGTGGACTGAAGGTCTCAATCGCACTGGAAACATCAAGGTCGCGCGCGGTCTCG<br>CCAGGATACATAGATTACCACAACCTCGAGCCCTCCACCCGCCGGAGACCCACGTAGTGAGTC |
| gBlock_U4U5    | GACTCAATTGCTTCTTAAGGCTCTCAATCGCAGTAAGGCTGGAGTTCGTAGACGGAAACATCAAGGTCGCGCCCTGCATTAGGATGATCTCGATCCCGCGAAATTAAATACGAC<br>TCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAAATAATTGGTTAACCTTAAGAAGGAGATATAACCATGGGAGATCTACTAGTAGCAGCCATC<br>ACCATCATCACCACAGCGGATCCGCTAGCTGACTGCAGAGAAGCTGGCCGCCCTCGAGTCGCTAGGCCGCTGAGCAATAACTAGCATAACCCCTGGGCC<br>CTCTAAACGGGTCTTGAGGGGTTTTGCTGAAAACCTCAGGACCGGGTGGACTGAAGGTCTCAATCGCACTGGAAACATCAAGGTCGCGCGCGGTCTCG<br>CCAGGATACATAGATTACCACAACCTCGAGCCCTCCACCCGCCGGAGACCCACGTAGTGAGTC |
| gBlock_U1U2_FL | CCCACAGCCAATTGACCTGCATGGCGCGCCATTACTCGCATCCATTCTCAGGCTGTCGTCGTCGCCGAATTGCCGCCCTCTAGAGTGGATCCAT<br>CATGGGTGCTAGCTAAAGCTTAGCTCGAGTGGTACCGTAGAGATCTGAACCTAGTAGCGGCCGCTGCAGCCGCTGGAGTTGTAGACGGAAACAAA<br>CGCAGAATCCAAGCCGCGCGCGTCTCGCAGGATACTAGATTACCACAACCTCGAGCCCTCCACCCGCCGGAGACCATGCATCCCACAGC  |
| gBlock_U2U3_FL | CCCACAGCCAATTGACCTGCATGGCGCGCCCTGGAGGTTCTGAGACGGAAACAAACGCAGAATCCAAGCCGGAATTGCCGCCCTCTAGAGTGGATCCAT<br>CCATGGGTGCTAGCTAAAGCTTAGCTCGAGTGGTACCGTAGAGATCTGAACCTAGTAGCGGCCGCTGCAGCCGCTGGAGTTGTAGACGGAAACAAA<br>GAAACACATCAAGGTGCGCGCGCGTCTCGCAGGATACTAGATTACCACAACCTCGAGCCCTCCACCCGCCGGAGACCATGCATCCCACAGC  |
| gBlock_U3U4_FL | CCCACAGCCAATTGACCTGCATGGCGCGCCCTGGAGGTTCTGAGACGGAAACAAACGCAGAATCCAAGCCGGAATTGCCGCCCTCTAGAGTGGATCCAT<br>CCATGGGTGCTAGCTAAAGCTTAGCTCGAGTGGTACCGTAGAGATCTGAACCTAGTAGCGGCCGCTGCAGCCGCTGGAGTTGTAGACGGAAACAAA<br>ACAACACGCAAAGTCCGCGCGCGTCTCGCAGGATACTAGATTACCACAACCTCGAGCCCTCCACCCGCCGGAGACCATGCATCCCACAGC   |
| gBlock_U4U5_FL | CCCACAGCCAATTGACCTGCATGGCGCGCCCTGACCTCCTGCCAGCAATTAGTAAGACAACACGCAAAGTCCGGAATTGCCGCCCTCTAGAGTGGATCCAT<br>CCATGGGTGCTAGCTAAAGCTTAGCTCGAGTGGTACCGTAGAGATCTGAACCTAGTAGCGGCCGCTGCAGCCGCTGGAGTTGTAGACGGAAACAAA<br>CAAGTCCGTTAGAGCGCGCGCGTCTCGCAGGATACTAGATTACCACAACCTCGAGCCCTCCACCCGCCGGAGACCATGCATCCCACAGC  |