Supporting Information

Rapid 40 kb Genome Construction From 52 Parts Through Data-Optimized Assembly Design

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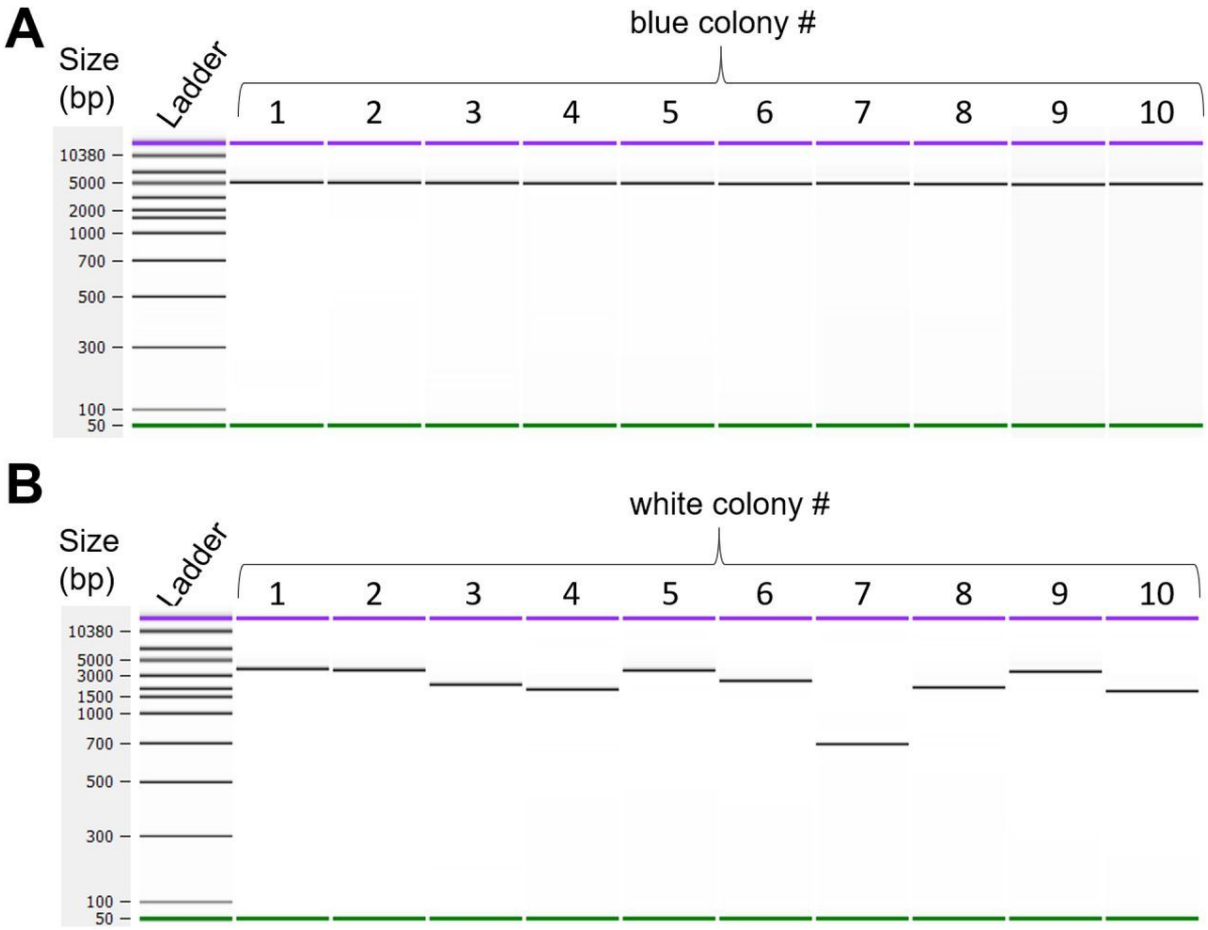


Figure S1. Verification of the 52-fragment *lac* operon cassette assembly.

Plasmid DNA was isolated from colonies using the Monarch Plasmid Miniprep kit and subjected to PCR with amplification primers that flank the desired insertion site. As anticipated, (A) blue colonies contained inserts of the expected size for correct assembly of all 52 fragments, and (B) white colonies harbored constructs carrying truncated assembly products.

Figure S2. Schematic of the T7 bacteriophage genome noting the location of SapI sites, BsmBI sites, NdeI sites, and the annealing location of the 8 primers used to verify the presence or absence of these sites in plaque analysis. The CDS annotated in GenBank for this genome are also shown. A GenBank file of the full genome with these annotations is also provided as part of the Supporting Materials.

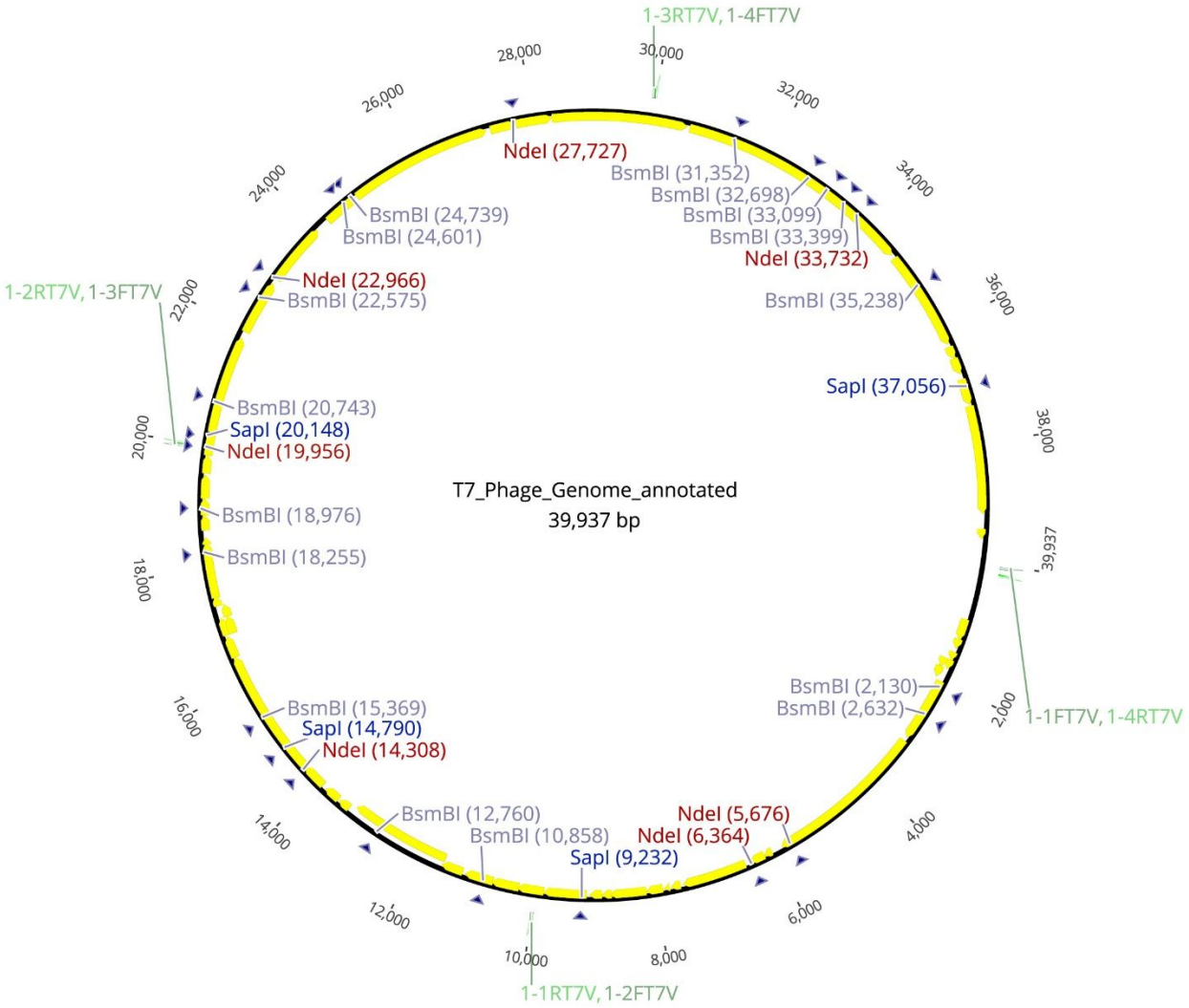
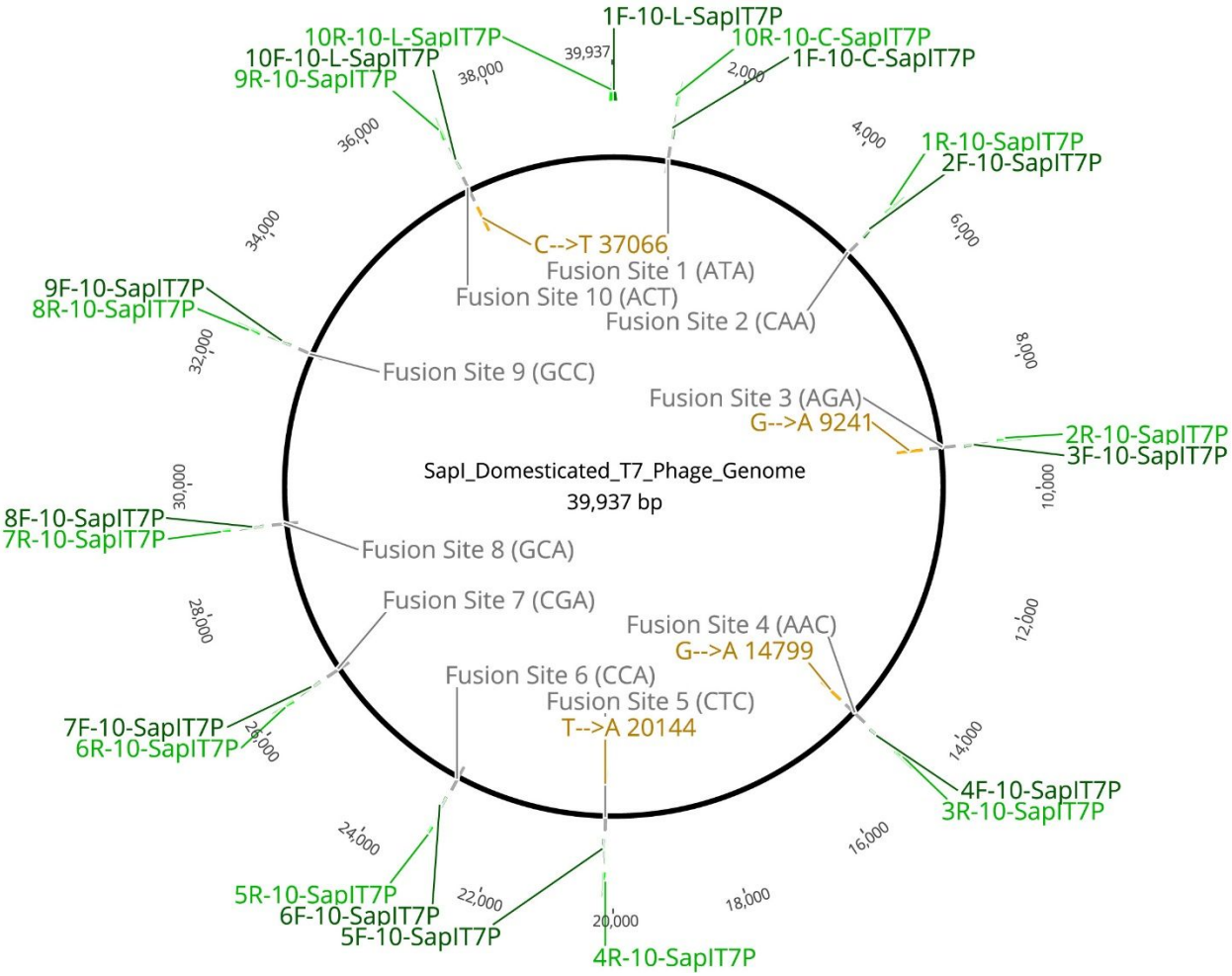


Figure S3. Schematic of the SapI 10 fragment assembly of the T7 phage genome. Fusion sites, sites of silent mutations used to remove native SapI recognition sites, and the binding location of all primers used to generate fragments ae shown. A GenBank file of the full genome with these annotations is also provided as part of the Supporting Materials.



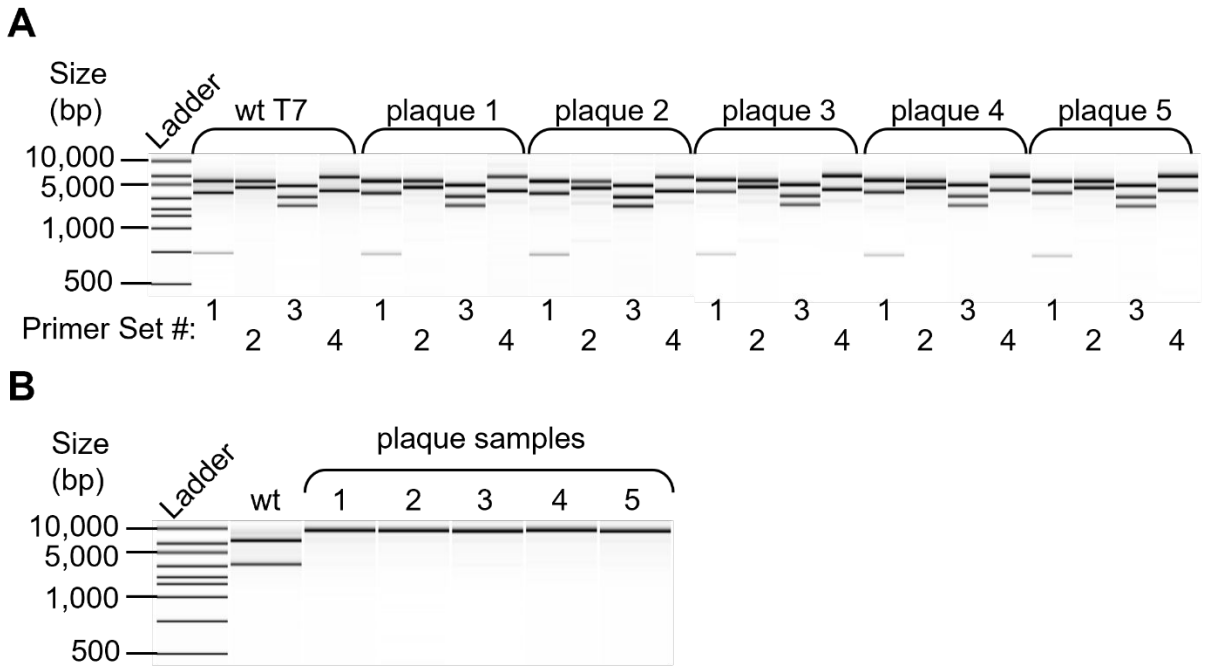
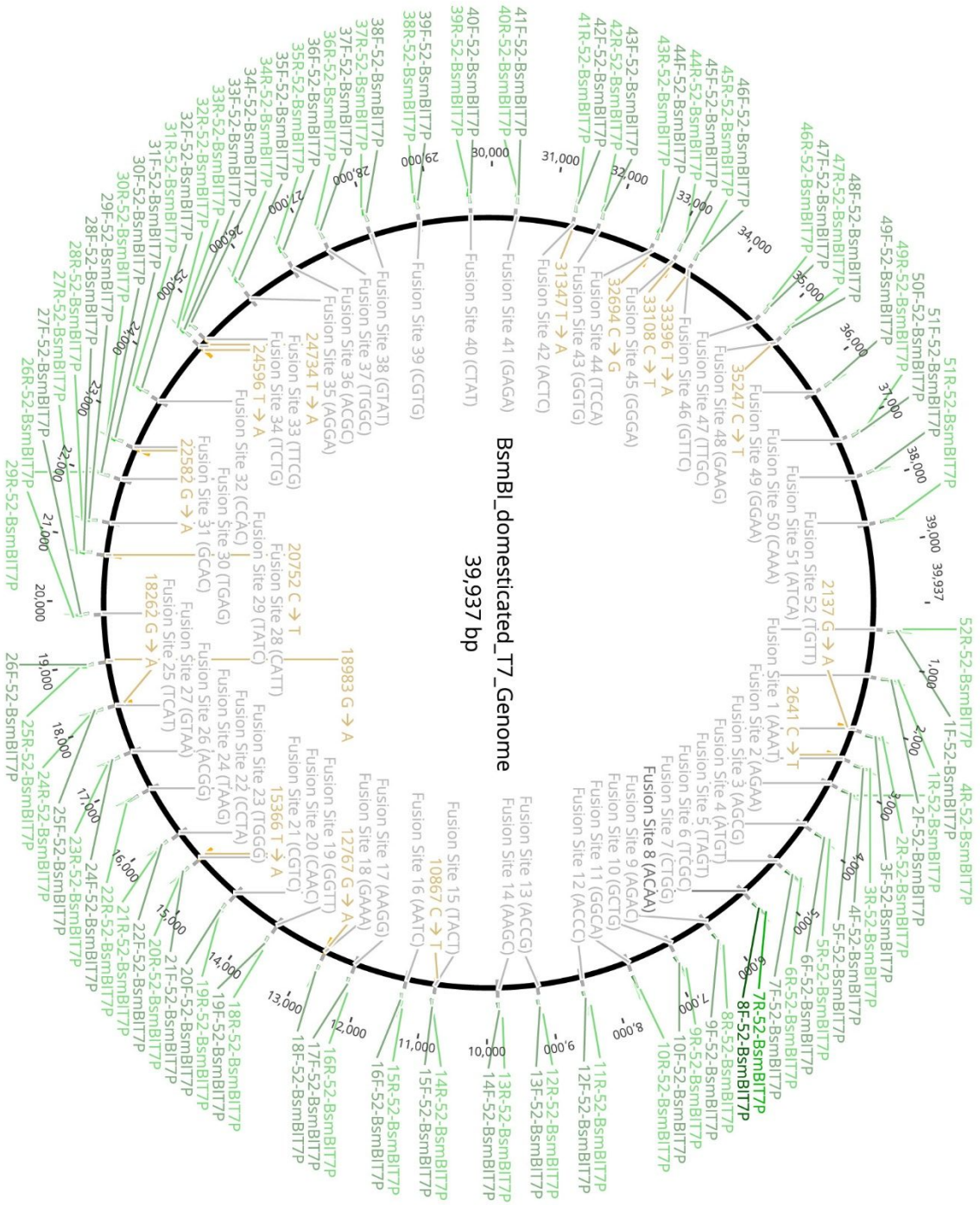


Figure S4. Verification of successful assembly of the T7 bacteriophage genome from 10-fragments. Plaque PCR was carried out using amplification primers (1-4) that span the 40 kb T7 phage genome (sequences shown in Table S4). Amplicons from five phage plaques (1-5) were compared to the parental wt T7 phage genome (wt) after restriction digest with NdeI. In all cases, the phage plaques produced a digestion pattern identical to the parental wt T7 phage. (B) To confirm that the assembled genomes harbored the desired silent mutations to remove native SapI restriction sites, we carried out amplicon digestion with SapI and showed that amplicon 4 from the parental T7 phage genome (wt) is sensitive to digestion by SapI, whereas amplicons from assembled genomes are inert to cleavage.

Figure S5. Schematic of the BsmBI 52 fragment assembly of the T7 phage genome. Fusion sites, sites of silent mutations used to remove native BsmBI recognition sites, and the binding location of all primers used to generate fragments are shown. A GenBank file of the full genome with these annotations is also provided as part of the Supporting Materials.



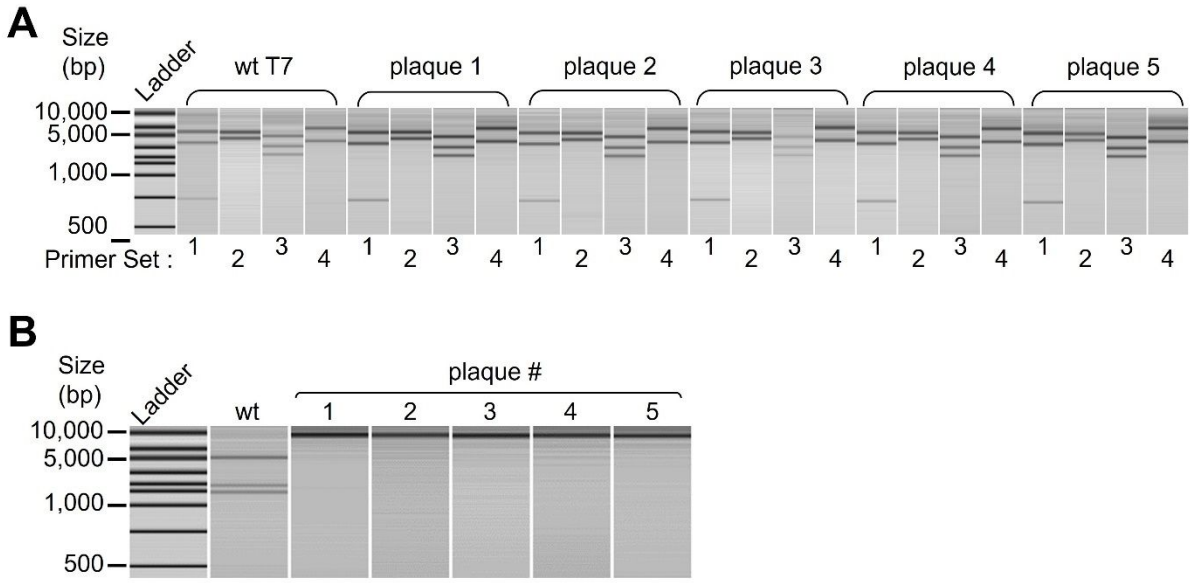


Figure S6. Verification of successful assembly of the T7 bacteriophage genome from 52-fragments. PCR amplification of genomic DNA from phage plaques was carried out using four sets of amplification primers (1-4) that span the T7 phage genome. Amplicons from 5 phage plaques (1-5) from the 52-fragment genome assembly reactions (90 cycles) were compared to the parental wt T7 phage genome (wt) after restriction enzyme digest with NdeI (A) or BsmBI (B). Comparison of amplicons digested with NdeI showed an identical digestion pattern across wt T7 phage and all assembled genome samples. Amplicon 4 from the parental T7 phage genome is sensitive to digest by BsmBI, but the same amplicon from the assembled genomes is inert to cleavage, indicating the successful introduction of the intended silent mutations into the phage genome.

Table S1. Overhang sequences for the 52-fragment *lac* cassette assembly reactions[a]

Number of Fragments	Overhang Sequences (5′ to 3′)
52	GGAG,CCAG,ATGT,TACA,GGCA,TATC,TAAG,CAGC,GAAC,CAAC,GCTT,TAGT,CTAT,GGAA,TTCG,AGAC,GTAT,GCGT,GATT,TTAC,TATT,TCGT,CAGA,GGGA,CTCA,GCAA,TGGA,CGTC,AACC,AGTA,TAGA,GAAA,AGGG,TTCT,ACAA,AGGT,TGTT,GAGT,TGGC,ACCG,ATTA,GTGC,AGCG,TCTT,CGTG,CCGA,ATCA,TCTC,CAAA,TTCA,TAGG,TATG,CCAT

Table S2. 52 fragment lac operon cassette assembly fragments and overhang sequences

Fragment #	Assembly Fragment Sequence (including Type IIS recognition sequence)
1	GGTCTCGGGAGTCATTTTTGACACCAGACCAACTGGTAATGGTAGCGACCGGCGCTCAGCTGGAATTCCGCCGATACTGACGGGCTCCAGCGAGACC
2	GGTCTCGCCAGGAGTCGTCGCCACCAATCCCCATATGGAAACCGTCGATATTCAGCCATGTGCCTTCTTCCGCGTGCAGCAGATGGCGATGGCTGGTTTCCATCAGTTGCTGTTGACTGTAGCGGCTGATGTCGAGACC
3	GGTCTCGATGTTGAACTGGAAGTCGCCGCGCCACTGGTGTGGGCCATAATTCAATTCGCGCGTCCCGCAGCGCAGACCGTTTTCGCTCGGGAAGACGTACGGGGTATACACGAGACC
4	GGTCTCGTACATGTCTGACAATGGCAGATCCCAGCGGTCAAAACAGGCGGCACGAGACC
5	GGTCTCGGGCAGTAAGGCGGTCGGGATAGTTTTCTTGCGGCCCTAATCCGAGCCAGTTTACCCGCTCTGCTACCTGCGCCAGCTGGCAGTTCAGGCCAATCCGCGCCGGATGCGGTGTATCCGAGACC
6	GGTCTCGTATCGCTCGCCACTTCAACATCAACGGTAATCGCCATTTGACCACTACCATCAATCCGGTAGGTTTTCCGGCTGATAAATAAGCGAGACC
7	GGTCTCGTAAGGTTTTCCCCTGATGCTGCCACGCGTGAGCGGTCGTAATCAGCACCGCATCAGCAAGTGTATCTGCCGTGCACTGCAACAACGCTGCTTCGGCCTGGTAATGGCCCGCCGCCTTCCAGCCGAGACC
8	GGTCTCGCAGCGTTCGACCCAGGCGTTAGGGTCAATGCGGGTCGCTTCACTTACGCCAATGTCGTTATCCAGCGGTGCACGGGTGAACCGAGACC
9	GGTCTCGGAACTGATCGCGCAGCGGCGTCAGCAGTTGTTTTTTATCGCCAATCCACATCTGTGAAAGAAAGCCTGACTGGCGGTTAAATTGCCAACCGAGACC
10	GGTCTCGCAACGCTTATTACCCAGCTCGATGCAAAAATCCATTTCGCTGGTGGTCAGATGCGGGATGGCGTGGGACGCGGCGGGGAGCGTCACACTGAGGTTTTCCGCCAGACGCCACTGCTGCCAGGCGCTGATGTGCCCGGCTTCGAGACC
11	GGTCTCGGCTTCTGACCATGCGGTCGCGTTCGGTTGCACTACGCGTACTGTGAGCCAGAGTTGCCCGGCGCTCTCCGGCTGCGGTAGTCGAGACC
12	GGTCTCGTAGTTCAGGCAGTTCAATCAACTGTTTACCTTGTGGAGCGACATCCAGAGGCACTTCACCGCTTGCCAGCGGCTTACCATCCAGCGCCACCATCCAGTGCAGGAGCTCGTTATCGCTATCGAGACC
13	GGTCTCGCTATGACGGAACAGGTATTCGCTGGTCACTTCGATGGTTTGCCCGGATAAACGGAACTGGAACGAGACC
14	GGTCTCGGGAAAAACTGCTGCTGGTGTTTTGCTTCCGTCAGCGCTGGATGCGGCGTGCGGTCGGCAAAGACCAGACCGTTCATACAGAACTGGCGATCGTTCGCGAGACC
15	GGTCTCGTTCGGCGTATCGCCAAAATCACCGCCGTAAGCCGACCACGGGTTGCCGTTTTCATCATATTTAATCAGCGACTGATCCACCCAGTCCCAGACCGAGACC
16	GGTCTCGAGACGAAGCCGCCCTGTAAACGGGGATACTGACGAAACGCCTGCCAGTATTTAGCGAAACCGCCAAGACTGTTACCCATCGCGTGGGCGTATCGAGACC
17	GGTCTCGGTATTCGCAAAGGATCAGCGGGCGCGTCTCTCCAGGTAGCGAAAGCCATTTTTTGATGGACCATTTCGGCACAGCCGGGAAGGGCTGGTCTTCATCCACGCGCGCGTCGAGACC

18	GGTCTCGGCGTACATCGGGCAAATAATATCGGTGGCCGTGGTGTCGGCTCCGCCGCCTTCATACTGCACCGGGCGG GAAGGATCGACAGATTTGATCCAGCGATACAGCGCGTCGTGATTCGAGACC
19	GGTCTCGGATTAGCGCCGTGGCCTGATTCATTCCCCAGCGACCAGATGATCACACTCGGGTGATTACGATCGCGCT GCACCATTCGCGTTACCGAGACC
20	GGTCTCGTTACGCGTTCGCTCATCGCCGGTAGCCAGCGCGGATCATCGGTCAGACGATTCATTGGCACCATGCCGT GGGTTTCAATATTCGAGACC
21	GGTCTCGTATTGGCTTCATCCACCACATACAGGCCGTAGCGGTCGCACAGCGTGTACCACAGCGGATGGTTCGGAT AATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATATCCTGCACCATCGTCGAGACC
22	GGTCTCGTCGTCTGCTCATCCATGACCTGACCATGCAGAGGATGATGCTCGTGACGGTTAACGCCTCGAATCAGCA ACGGCTTGCCGTTCAGCAGCAGCAGACGAGACC
23	GGTCTCGCAGACCATTTTCAATCCGCACCTCGCGGAAACCGACATCGCAGGCTTCTGCTTCAATCAGCGTGCCGTC GGCGGTGTGCAGTTCAACCACCGCACGATAGAGATTCGGGACGAGACC
24	GGTCTCGGGGATTTCGGCGCTCCACAGTTTCGGGTTTTCGACGTTCAGACGTAGTGTGACGCGATCGGCATAACCA CCACGCTCACGAGACC
25	GGTCTCGCTCATCGATAATTTCACCGCCGAAAGGCGCGGTGCCGCTGGCGACCTGCGTTTCACCCTGCCATAAAGA AACTGTTACCCGTAGGTAGTCACGCAACGAGACC
26	GGTCTCGGCAACTCGCCGCACATCTGAACTTCAGCCTCCAGTACAGCGCGGCTGAAATCATCATTAAAGCGAGTGG CAACATGGACGAGACC
27	GGTCTCGTGGAAATCGCTGATTTGTGTAGTCGGTTTATGCAGCAACGAGACGTCACGGAAAATGCCGCTCATCCGC CACATATCCTGATCTTCCAGATAACTGCCGTCCGAGACC
28	GGTCTCGCGTCACTCCAGCGCAGCACCATCACCGCGAGGCGGTTTTCTCCGGCGCGTAAAAATGCGCTCAGGTCAA ATTCAGACGGCAAACGACTGTCCTGGCCGTAACCCGAGACC
29	GGTCTCGAACCGACCCAGCGCCCGTTGCACCACAGATGAAACGCCGAGTTAACGCCATCAAAAATAATTCGCGTCT GGCCTTCCTGTAGCCAGCTTTCATCAACATTAAATGTGAGCGAGTACGAGACC
30	GGTCTCGAGTAACAACCCGTCGGATTCTCCGTGGGAACAAACGGCGGATTGACCGTAATGGGATAGGTCACGTTG GTGTAGACGAGACC
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32	GGTCTCGGAAACCAGGCAAAGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCC TCTTCGCTATTACGCCAGCTGGCGAAAGGGCGAGACC
33	GGTCTCGAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAAC GACGGCCAGTGAATCCGTAATCATGGTCATATGTATATCTCCTTCTCGAGACC
34	GGTCTCGTTCTTAAAGTTAAACAAAATTATTTCTAGAGGGGAATTGTTATCCGCTCACAATTCCACACAACGAGAC C
35	GGTCTCGACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGATCGAGATCTCGATCCTCTACGCCGGACG CATCGTGGCCGGCATCACCGGCGCCACAGGTCGAGACC

36	GGTCTCGAGGTGCGGTTGCTGGCGCCTATATCGCCGACATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCT CATGAGCGCTTGTTTCGGCGTGGGTATGGTGGCAGGCCCCGTGGCCGGGGGACTGTTCGAGACC
37	GGTCTCGTGTTGGGCGCCATCTCCTTGCATGCACCATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACT GGGCTGCTTCCTAATGCAGGAGTCGAGACC
38	GGTCTCGGAGTCGCATAAGGGAGAGCGTCGAGATCCCGGACACCATCGAATGGCGCAAAACCTTTCGCGGTATGG CCGAGACC
39	GGTCTCGTGGCATGATAGCGCCCGGAAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTATACGAT GTCGCAGAGTATGCCGGTGTCTCTTATCAGACCGCGAGACC
40	GGTCTCGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGC GATGGCGGAGCTGAATTACGAGACC
41	GGTCTCGATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCT CCAGTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCGAGAC C
42	GGTCTCGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGCGAGACC
43	GGTCTCGAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGATGACCA GGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTCGAGACC
44	GGTCTCGTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGC GTGCGAGACC
45	GGTCTCGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTC GGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGACGAGACC
46	GGTCTCGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAA TGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCACGAGACC
47	GGTCTCGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCCG AGACC
48	GGTCTCGTCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAAC AGGATTTTCGCCTGCTGGGGCAAACGAGACC
49	GGTCTCGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCC GTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAC GAGACC
50	GGTCTCGTTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAAT GTAAGTTAGCTCACTCATTAGGCGAGACC
51	GGTCTCGTAGGCACCGGGATCTCGACCGATGCCCTTGAGAGCCTTCAACCCAGTCAGCTCCTTCCGGTGGGCGCGG GGCATGACTATCGTCGCCGCACTTATGCGAGACC
52	GGTCTCGTATGACTGTCTTCTTTATCATGCAACTCGTAGGACAGGTGCCGGCAGCGCTCTGGGTCATTTTCGGCGAG GACCCATCGAGACC

Table S3. PCR primer sequences

Name	Description	Sequence (5′ to 3′)
pUC57mini-1	Forward Amplification primer for pre-cloned lac cassette assembly fragments	GGGTTCCGCGCACATTTC
pUC57mini-2	Reverse Amplification primer for pre-cloned lac cassette assembly fragments	TTTGCTGGCCTTTTGCTCACAT
1F-10-L-SapIT7P	Forward Primer: Fragment #1 of 10-part T7 gDNA Linear Assembly	TCTCACAGTGTACGGACC
1F-10-C-SapIT7P	Forward Primer: Fragment #1 of 10-part T7 gDNA Circle Assembly	AACAGCGCTCTTCCATAATGCAGTTCCGCACTAC
2F-10-SapIT7P	Forward Primer: Fragment #2 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCCAAGCTGGGCACTAAGGCACTG
3F-10-SapIT7P	Forward Primer: Fragment #3 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCAGAACGTGGCTTTGGGAAC
4F-10-SapIT7P	Forward Primer: Fragment #4 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCAACAGGGTGAAGAATACG
5F-10-SapIT7P	Forward Primer: Fragment #5 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCCTCATCAACCTTAAGCCACCGC
6F-10-SapIT7P	Forward Primer: Fragment #6 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCCCACTTCTCGCCACATG
7F-10-SapIT7P	Forward Primer: Fragment #7 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCCGAGGTCCAGCTTCACGTC
8F-10-SapIT7P	Forward Primer: Fragment #8 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCGCAGTATCGCTTGAAGATTAAC
9F-10-SapIT7P	Forward Primer: Fragment #9 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCGCCACAAAGGTACTTACG
10F-10-L-SapIT7P	Forward Primer: Fragment #10 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCACTCAAAGAGCAATCGATGC
1R-10-SapIT7P	Reverse Primer: Fragment #1 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCTTGACTTTCTCAGAGATTTCACC
2R-10-SapIT7P	Reverse Primer: Fragment #2 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCTCTTCGTTGCCGTAGTCC
3R-10-SapIT7P	Reverse Primer: Fragment #3 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCGTTCTTCAAGCATACGCTTAAAG
4R-10-SapIT7P	Reverse Primer: Fragment #4 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCGAGCGTATAGCGAGAAC
5R-10-SapIT7P	Reverse Primer: Fragment #5 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCTGGTCACGGAGGTACGAG
6R-10-SapIT7P	Reverse Primer: Fragment #6 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCTCGGACTAGCAAAGTAGAC
7R-10-SapIT7P	Reverse Primer: Fragment #7 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCTGCTCGTTCAGCTTCGC
8R-10-SapIT7P	Reverse Primer: Fragment #8 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCGGCGACTCTCAGGGAAG
9R-10-SapIT7P	Reverse Primer: Fragment #9 of 10-part T7 gDNA Assembly	AACAGCGCTCTTCCAGTACTCTTCGCAGCCTC
10R-10-L-SapIT7P	Reverse Primer: Fragment #10 of 10-part T7 gDNA Linear Assembly	AGGGACACAGAGAGACAC
10R-10-C-SapIT7P	Reverse Primer: Fragment #10 of 10-part T7 gDNA Circle Assembly	AACAGCGCTCTTCCTATCGGCAGCCATGTGAATAG
1-1FT7V	Forward T7 phage genome analysis primer (Set 1)	TCTGTCTCTCACAGTGTACGGACCTAAAGTTC
1-2FT7V	Forward T7 phage genome analysis primer (Set 2)	TCTGTCAGCAATACCGGAAAGGTTGTC

1-3FT7V	Forward T7 phage genome analysis primer (Set 3)	TCTGTCATGAGGAGACATATGGTCCAG
1-4FT7V	Forward T7 phage genome analysis primer (Set 4)	TCTGTCCCGACTGACTGTTAAGCGGTC
1-1RT7V	Reverse T7 phage genome analysis primer (Set 1)	TCTGTCGGAGCATTAGACATTACGCGATGAC
1-2RT7V	Reverse T7 phage genome analysis primer (Set 2)	TCTGTCATATGCATCACCACAATTCCTGTTG
1-3RT7V	Reverse T7 phage genome analysis primer (Set 3)	TCTGTCTTTATCATCCTCAAAGCGTTGC
1-4RT7V	Reverse T7 phage genome analysis primer (Set 4)	TCTGTCAGGGACACAGAGAGACACTCAAG
1F-52-BsmBIT7P	Forward Primer: Fragment #1 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAAATTTATCAAAAAGAGTATTGACTT
2F-52-BsmBIT7P	Forward Primer: Fragment #2 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAGAAGACTTGCTCAATGAATACT
3F-52-BsmBIT7P	Forward Primer: Fragment #3 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAGCGAAACGTGTGATGG
4F-52-BsmBIT7P	Forward Primer: Fragment #4 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCATGTACCATACATCACCGACCCG
5F-52-BsmBIT7P	Forward Primer: Fragment #5 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTAGTCTTATCTTACAGGTCATCT
6F-52-BsmBIT7P	Forward Primer: Fragment #6 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTCGCACCTGAATACGCTGAG
7F-52-BsmBIT7P	Forward Primer: Fragment #7 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCTGGAGAACACTTGGTGGGCTGA
8F-52-BsmBIT7P	Forward Primer: Fragment #8 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCACAAGAAGCCTATTCAGACGC
9F-52-BsmBIT7P	Forward Primer: Fragment #9 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAGACTTAGCGGTCATTTATG
10F-52-BsmBIT7P	Forward Primer: Fragment #10 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGCTGCACACTGGACACCTTCAC
11F-52-BsmBIT7P	Forward Primer: Fragment #11 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGGCAGCTATCCTGACGCTTGC
12F-52-BsmBIT7P	Forward Primer: Fragment #12 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCACCCACGTAAACCACATCTGAAT
13F-52-BsmBIT7P	Forward Primer: Fragment #13 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCACCGCTGAACCTTACGCTTACAT
14F-52-BsmBIT7P	Forward Primer: Fragment #14 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAAGCAGACGAAGACGGAGACT
15F-52-BsmBIT7P	Forward Primer: Fragment #15 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTACTGTGGAGGCAGGACG
16F-52-BsmBIT7P	Forward Primer: Fragment #16 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAATCTCCGTCTGGCTCTCCC
17F-52-BsmBIT7P	Forward Primer: Fragment #17 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAAGGTACGAGTGGCAGTTCT
18F-52-BsmBIT7P	Forward Primer: Fragment #18 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGAAACGGATAGACTGCTCGCT
19F-52-BsmBIT7P	Forward Primer: Fragment #19 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGGTTTACCGCTGGAAGGACTCTA
20F-52-BsmBIT7P	Forward Primer: Fragment #20 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCAACGGTCACAAGTATGAC
21F-52-BsmBIT7P	Forward Primer: Fragment #21 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCGTCACGTGACCACATTCAGAAG
22F-52-BsmBIT7P	Forward Primer: Fragment #22 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCCTACCCGAGATAACGCTAAGAC
23F-52-BsmBIT7P	Forward Primer: Fragment #23 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTGGGACGAAGTAAAAGGTAAGAC
24F-52-BsmBIT7P	Forward Primer: Fragment #24 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTAAGAATCAACTAATGGAAGCTG

25F-52-BsmBIT7P	Forward Primer: Fragment #25 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTCATGAAACGCTTTGGGACTGC
26F-52-BsmBIT7P	Forward Primer: Fragment #26 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCACGGATACCGAGGGCACCG
27F-52-BsmBIT7P	Forward Primer: Fragment #27 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGTAAGAAATCCTTGAGTGTAGCC
28F-52-BsmBIT7P	Forward Primer: Fragment #28 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCATTCGGCAACGTTCTGCAAAT
29F-52-BsmBIT7P	Forward Primer: Fragment #29 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTATCGAGGCTCGCCTTTCGTTT
30F-52-BsmBIT7P	Forward Primer: Fragment #30 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTGAGGAACATGAGCAGAACA
31F-52-BsmBIT7P	Forward Primer: Fragment #31 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGCACAACCCTGAGGCTG
32F-52-BsmBIT7P	Forward Primer: Fragment #32 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCCACTCAGAACAAGG
33F-52-BsmBIT7P	Forward Primer: Fragment #33 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTTCGACTCCGCGACTACGATG
34F-52-BsmBIT7P	Forward Primer: Fragment #34 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTCTGCATGGAGTATGAGATGGAC
35F-52-BsmBIT7P	Forward Primer: Fragment #35 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAGGACCAAGTTCTATGGGAAACC
36F-52-BsmBIT7P	Forward Primer: Fragment #36 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCACGCCATTGACTTACAGGGAGA
37F-52-BsmBIT7P	Forward Primer: Fragment #37 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTGGCTCGAAATTAATACGACTCA
38F-52-BsmBIT7P	Forward Primer: Fragment #38 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGTATGCTTGAAGGTTCCTC
39F-52-BsmBIT7P	Forward Primer: Fragment #39 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCGTGGTTTCAACGGGGACAT
40F-52-BsmBIT7P	Forward Primer: Fragment #40 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCTATGAAGTTGAAGTACCTTC
41F-52-BsmBIT7P	Forward Primer: Fragment #41 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGAGAACCAGAAGAAACTCGAAGA
42F-52-BsmBIT7P	Forward Primer: Fragment #42 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCACTCGATAATGGTTTTGATGTGT
43F-52-BsmBIT7P	Forward Primer: Fragment #43 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGGTGGCTTTGTGTTTGGCG
44F-52-BsmBIT7P	Forward Primer: Fragment #44 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTCCAGACCTGAGGTCAAGGCC
45F-52-BsmBIT7P	Forward Primer: Fragment #45 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGGGAGATGGTAAGAAGACTGGC
46F-52-BsmBIT7P	Forward Primer: Fragment #46 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGTTCGGGAAGGAGGTG
47F-52-BsmBIT7P	Forward Primer: Fragment #47 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTTGCTACACGTACTACTATCTCT
48F-52-BsmBIT7P	Forward Primer: Fragment #48 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGAAGCCAAGCGGTTCAAG
49F-52-BsmBIT7P	Forward Primer: Fragment #49 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGGAAAGACGTGTAGTCCACGGAT
50F-52-BsmBIT7P	Forward Primer: Fragment #50 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCAAAACTACCGGAACCTC
51F-52-BsmBIT7P	Forward Primer: Fragment #51 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCATCATTGCGGATGACGTTGAGAT
52F-52-BsmBIT7P	Forward Primer: Fragment #52 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTGTTCGGTAAGGTATTCAG
1R-52-BsmBIT7P	Reverse Primer: Fragment #1 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTTCTGCGTCTTCCC
2R-52-BsmBIT7P	Reverse Primer: Fragment #2 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCGCTGTTGACCATCTCG

3R-52-BsmBIT7P	Reverse Primer: Fragment #3 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCACATCTCCATTTGAGAACATGAT
4R-52-BsmBIT7P	Reverse Primer: Fragment #4 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCACTATCAGCCCATTAACATTGCG
5R-52-BsmBIT7P	Reverse Primer: Fragment #5 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGCGAGTTCGATAGTCTCAGAG
6R-52-BsmBIT7P	Reverse Primer: Fragment #6 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCCAGTGGAGACTTAGCGCAAG
7R-52-BsmBIT7P	Reverse Primer: Fragment #7 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTTGTATTCCTGCCACACAGGGAA
8R-52-BsmBIT7P	Reverse Primer: Fragment #8 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGTCTAACTGGAACAGCTTGTTTG
9R-52-BsmBIT7P	Reverse Primer: Fragment #9 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCAGCTTAAAGGGAACTTTATCTT
10R-52-BsmBIT7P	Reverse Primer: Fragment #10 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTGCCAAAGCCGCAAGGAAT
11R-52-BsmBIT7P	Reverse Primer: Fragment #11 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGGGTCCATCGCTCGGATTTC
12R-52-BsmBIT7P	Reverse Primer: Fragment #12 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCGGTACCCAGCGCAG
13R-52-BsmBIT7P	Reverse Primer: Fragment #13 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGCTTCCTCGGACTCTTCGTCGTC
14R-52-BsmBIT7P	Reverse Primer: Fragment #14 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAGTACCATCTCGCTTGATGATAA
15R-52-BsmBIT7P	Reverse Primer: Fragment #15 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGATTGTCTAAGGCGTCGTTG
16R-52-BsmBIT7P	Reverse Primer: Fragment #16 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCCTTACCAGCAGGTAGAACCT
17R-52-BsmBIT7P	Reverse Primer: Fragment #17 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTTTCAGCCTCGGCGAATGAG
18R-52-BsmBIT7P	Reverse Primer: Fragment #18 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAACCATAGTCGCCTTACCG
19R-52-BsmBIT7P	Reverse Primer: Fragment #19 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGTTGTGGAACACAATAAGACCG
20R-52-BsmBIT7P	Reverse Primer: Fragment #20 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGACGAAGGGTTAAACACAA
21R-52-BsmBIT7P	Reverse Primer: Fragment #21 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTAGGTAGTTCAGCAGCTATCTGG
22R-52-BsmBIT7P	Reverse Primer: Fragment #22 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCCCATGTGAAAATAAGCACATCT
23R-52-BsmBIT7P	Reverse Primer: Fragment #23 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCTTAAGGCTATCGTTCTCAC
24R-52-BsmBIT7P	Reverse Primer: Fragment #24 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCATGAGGCTCAGGGTC
25R-52-BsmBIT7P	Reverse Primer: Fragment #25 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCCGTTTCATCAGAAGACCCACC
26R-52-BsmBIT7P	Reverse Primer: Fragment #26 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTTACCGCCAGCACGAGC
27R-52-BsmBIT7P	Reverse Primer: Fragment #27 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAATGCATCTCGTTGGACCACA
28R-52-BsmBIT7P	Reverse Primer: Fragment #28 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGATAGCGTCACTTACGGCTTTAG
29R-52-BsmBIT7P	Reverse Primer: Fragment #29 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCTCAACGGAACCACCAGACATCA
30R-52-BsmBIT7P	Reverse Primer: Fragment #30 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGTGCGTTTCAAGGTGGTTAT
31R-52-BsmBIT7P	Reverse Primer: Fragment #31 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGTGGTCTCAATTACGGTAGCAGT
32R-52-BsmBIT7P	Reverse Primer: Fragment #32 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCGAATAATGTTCACAGTAATACC

33R-52-BsmBIT7P	Reverse Primer: Fragment #33 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCAGAGTCGTCTAGCCTCATCTTC
34R-52-BsmBIT7P	Reverse Primer: Fragment #34 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTCCTCAGTGTTCCAACCTAAAGT
35R-52-BsmBIT7P	Reverse Primer: Fragment #35 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGCGTTCTTAGTGAAAGAGATTCT
36R-52-BsmBIT7P	Reverse Primer: Fragment #36 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGCCACCACAGGGAGAATA
37R-52-BsmBIT7P	Reverse Primer: Fragment #37 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCATACTCTCTCCGATAGCC
38R-52-BsmBIT7P	Reverse Primer: Fragment #38 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCACGCTGATAATCAACGTCC
39R-52-BsmBIT7P	Reverse Primer: Fragment #39 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCATAGCATCCTTGGCACCGTCT
40R-52-BsmBIT7P	Reverse Primer: Fragment #40 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTCTCACTCCAGACCTTC
41R-52-BsmBIT7P	Reverse Primer: Fragment #41 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGAGTCGACCAGCACGGAA
42R-52-BsmBIT7P	Reverse Primer: Fragment #42 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCACCTAAGGCAGCACC
43R-52-BsmBIT7P	Reverse Primer: Fragment #43 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTGGACACGTAGCTGTTCATCC
44R-52-BsmBIT7P	Reverse Primer: Fragment #44 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTCCCTCAGCTTTCGCTTTGAGA
45R-52-BsmBIT7P	Reverse Primer: Fragment #45 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGAACAGTGACGCATGGAGTTCC
46R-52-BsmBIT7P	Reverse Primer: Fragment #46 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCGCAAAGCGATAGTCTGTATTA
47R-52-BsmBIT7P	Reverse Primer: Fragment #47 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCCTTCATCTCGGAAACCCTTGG
48R-52-BsmBIT7P	Reverse Primer: Fragment #48 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTTCCTTGTGATTTACCAATTACT
49R-52-BsmBIT7P	Reverse Primer: Fragment #49 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTTTGACTCTGACGCGCAACC
50R-52-BsmBIT7P	Reverse Primer: Fragment #50 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCTGATAATGTCAGCACGGCTA
51R-52-BsmBIT7P	Reverse Primer: Fragment #51 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCAACATACCGTCACCGAAGT
52R-52-BsmBIT7P	Reverse Primer: Fragment #52 of 52-part T7 gDNA Assembly	TCACGGCGTCTCCATTTTAAATTAATCTTTTAAGTCTCTTT

Table S4. Lactose Operon Cassette Assembly from 52-fragments

Fragment Lot[a]	Assembly Protocol[b]	%Correct[c]	Yield[d]
Lot 1	37°C/16°C cycling	19 ± 2	270 ± 40
Lot 1	37°C static	49 ± 2	727 ± 160
Lot 2	37°C/16°C cycling	10 ± 3	4 ± 2
Lot 2	37°C static	16 ± 2	12 ± 1

[a]To analyze the reproducibility of the assembly results, we generated all 52-fragments in two separate lots (Lot 1 and Lot 2) prepared several months apart.

[b]Reactions to reconstitute the *lac* operon cassette were incubated for 48 h at 37°C or at 37°C/16°C using an oscillating thermocycling protocol (5 minutes at each temperature) and then subjected to a final heat-soak step at 60°C for 5 minutes before being incubated at 4°C prior to transformation. Importantly, we anticipated that the 37°C static protocol would be significantly less efficient due to suboptimal ligation at 37°C; thus, we carried out extended incubation reactions (48 hours).

[c]The percentage of transformants harboring correctly assembled constructs is shown. Experiments were carried out with at least 2 experimental replicates, with the standard deviation from the mean shown.

[d]The yield of colony-forming units harboring correctly assembled constructs per 100 microliters of assembly reaction transformed into E. coli cells is shown. At least two experimental replicates were carried out for every assembly reaction, with the standard deviation from the mean shown.

Table S5. Silent mutations to permit T7 phage genome assembly with SapI

Location	Mutation	Coding Sequence
9241	G → A	ssDNA binding protein
14799	G → A	DNA polymerase
20144	T → A	unnamed protein product
37066	C → T	homology lambda lys Rz

Table S6. Overhang sequences for the T7 phage genome assembly reactions

Number of Fragments	Fusion Sites Sequence(genomic location)
10	ATA (1052..1054), CAA (4997..4999), AGA (9238..9240), AAC (14798..14800), CTC (20141..20143), CCA (23103..23105), CGA (26229..26231), GCA (29245..29247), GCC (32573..32575), ACT (37067..37069)
52	AAAT (443..446), AGAA (1218..1221), AGCG (2132..2135), ATGT (2640..2543), TAGT (3101..3104), TCGC (3907..3910), CTGG (4659..4662), ACAA (5386..5389), AGAC (6202..6205), GCTG (6888..6891), GGCA (7643..7646), ACCC (8452..8455), ACCG (9191..9194), AAGC (9831..9834), TACT (10870..10873), AATC (11360..11363), AAGG (12243..12246), GAAA (12765..12768), GGTT (13679..13682), CAAC (14529..14532), CGTC (15362..15365), CCTA (15898..15901), TGGG (16774..16777), TAAG (17448..17451), TCAT (18256..18259), ACGG (18984..18987), GTAA (19785..19785), CATT (20754..20757), TATC (21280..21283), TGAG (22021..22024), GCAC (22585..22588), CCAC (23466..23469), TTCG (24592..24595), TCTG (24736..24739), AGGA (25740..25743), ACGC (26583..26586), TGGC (27246..27249), GTAT (27990..27993), CGTG (28817..28820), CTAT (29655..29658), GAGA (30404//30407), ACTC

	(31347..31350), GGTG (31771..31774), TCCA (32695..32698), GGGA (33102..33105), GTTC (33399..33402), TTGC (34778..34781), GAAG (35248..35251), GGAA (36300..36303), CAAA (37189..37192), ATCA (37838..37841), TGTT (38649..38652)

Table S7. Frequency of Type IIS recognition sites in T7 bacteriophage genome

Type IIS Restriction enzyme[1]	Number of Recognition Sites
SapI (BspQI, LguI)	4
PaqCI (AarI)	5
BsmBI (Esp3I)	16
BsaI (Eco31I)	29
BbsI (BpiI)	38

[1]Isoschizomer(s) are shown in brackets

Table S8. Silent mutations to permit T7 phage genome assembly with BsmBI

Location	Mutation	Coding Sequence
2137	G → A	protein kinase
2614	C → T	protein kinase
10867	C → T	lysozyme
12767	G → A	primase/helicase
15366	T → A	DNA polymerase
18262	G → A	exonuclease
18983	G → A	unnamed protein product
20752	C → T	head-tail connector
22582	G → A	scaffolding protein
24596	T → A	tail protein
24734	T → A	tail protein
31347	T → A	internal virion
32694	C → G	internal virion
33108	C → T	internal virion
33396	T → A	internal virion
35247	C → T	tail fiber protein

Table S9. SNPs differentiating our in-lab T7 strain from the Genbank strain.

Location	Mutation	Coding Sequence
1895	G → GA	unnamed protein product
6238	C → T	unnamed protein product
10001	C → T	unnamed protein product
30809	G → A	internal virion

Table S10. Novel SNPs from reconstituted phage genomes identified by nanopore sequencing

Plaque #	Location	Mutation	Coding Sequence
2	7609	T → G	unnamed protein product
6	14378	A → G	DNA polymerase
9	26773	G → T	tail protein