Title: Directed evolution of colE1 plasmid replication compatibility: a fast tractable tunable model for investigating biological orthogonality.

Authors:

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Supplementary information:

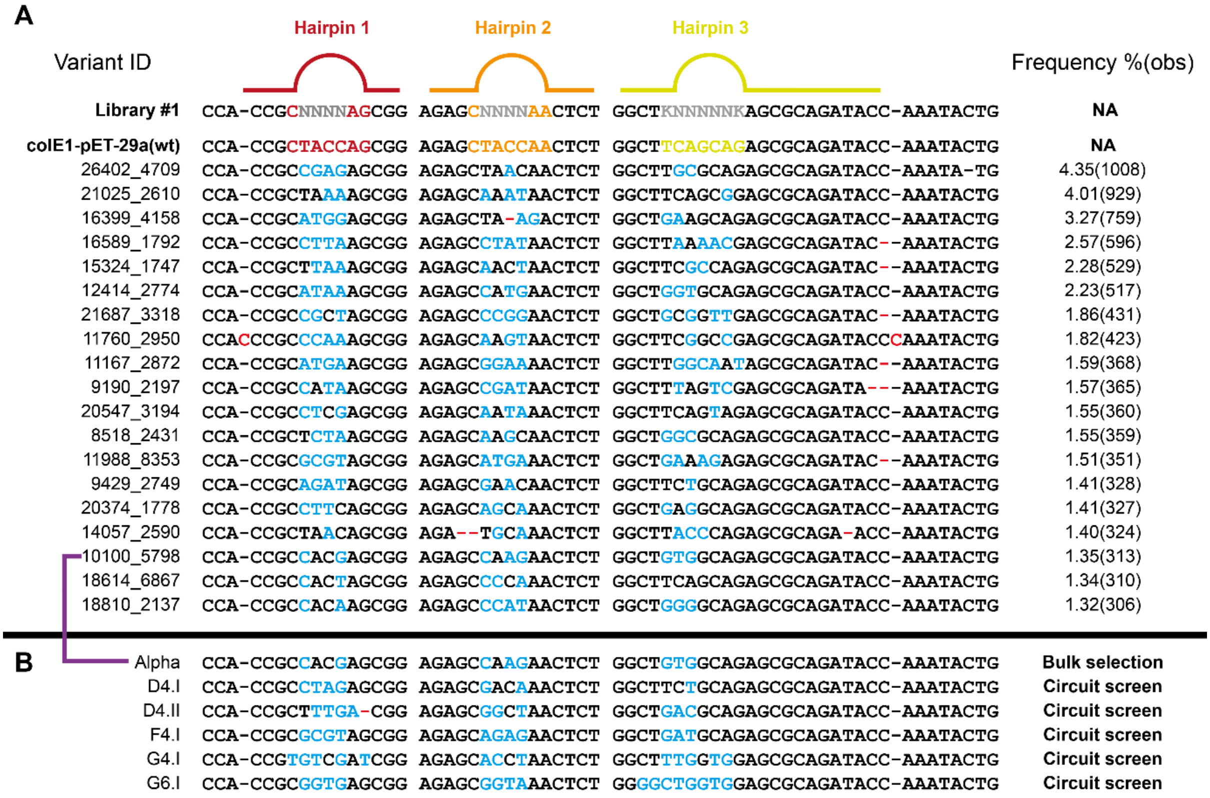
Primer name	Primer sequence
Construct and library assembly	
ES VCTR FW	AAAGGTCTCAAGTGTAGCCGTAGTTAGGC
ES VCTR RV	AAAGGTCTCACAAGCAGCAGATTACGCG
ES ULTR RNAI FW	GGGGGTCTCACACTTAGAAG
ES ULTR REV	ACACACAGGTCTCACTTGC
ES COLEI ins GIBS FW	CTACGCATGGCTCAAAACACCCCTTGT
ES COLEI ins GIBS RV	TTTTTCCATAGGCTCCGCC
ES 002 FW	AAAGGTCTCACGTTAAAGGAAGCTGAGTTGGCT
ES 002 RV	AAAGGTCTCATAGAGGGGAATTGTTATCCGC
ES 003 FW	AAAGGTCTCATCTAGGGCTAACAGGAGGAATTAAC
ES 003 RV	AAAGGTCTCAAACGCATCCGCCAAAACAGC
ES BAD GFP FW	AAAGGTCTCACCCGTTTTTTGGGCTAAC
ES BAD GFP RV	AAAGGTCTCAGCTTCGCTTCTGCGTTCTGAT
ES PET GFP FW	AAAGGTCTCAAAGCCCGAAAGGAA
ES PET GFP RV	AAAGGTCTCACGGGAATTGTTATCCGCT
ES sfGFP plasm ampl FW	GGCGGAGCCTATGGAAAAA
ES sfGFP plasm ampl RV	GGGGTGTTTTGAGCCATGCGTAGAGGATCTGCTCA
SC_pBAD_dOri_FW	AAACGTCTCACTTGCATGTGTCAGAGGTTTTCAC
SC_pBAD_dOri_RV	AAACGTCTCATCACTCAGTGGAACGAAAACTCAC
SC_pBAD_dOri_dATB_RV	AAACGTCTCATCACTGTAGAAACGCAAAAAGGCC
SC_pET_addOri_addATB_FW	AAAGGTCTCAGTGACGTTTACAATTTCAGGTGGC
SC_pET_addOri_addATB_RV	AAAGGTCTCACAAGATCAGCTCACTCAAAGGC
SC_pWH_addOri_addATB_FW	AAAGGTCTCAGTGATTCCGTGATGGTAACTTCAC
SC_pWH_addOri_FW	AAAGGTCTCAGTGAGCAAGGATCTTCTTGAGATCC
SC_pWH_addOri_addATB_RV	AAAGGTCTCACAAGAATCATCTGGCCATTCGATG
AR_pWHalpha_Fw	TATGGAAAAACGCCAGCAACG
AR_pWHalpha_Rv	AAGATCCTTGCACTCGAGTTGATCG
VP023F	TTTGGTCTCA AAGTTGCACTCGAGTTGATCGGGC
VP023R	TTTGGTCTCA TTCCGCCTTTTTACGGTTCCTGGCC
WH81	TCCTCGAGGCTTGGATTCTC
WH82	TGCACTCGAGTTGATCGGG
WH83	TGCCCGATCAACTCGAGTGCAAGGATCTTCTTGAGATCC
WH84	AACGAGACATCATTTTTTGCCCTCGTTATCTAG
WH85	AGGGCAAAAAATGATGTCTCGTTTAGATAAAAG
WH86	AGAATCCAAGCCTCGAGGAAGATCCTTTGATCTTTTCTAC
WH87	TCCCTATCAGTGATAGAGAACCTCTAGAAATAATTTTGTTTAAC
WH88	ATCAATGATAGAGTGTCAACATTTCGCGGGATCGAG
TetA	GTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCAGTGATAGAGAA
TetAR	TTCTCTATCACTGATAGGGAGTGGTAAAATAACTCTATCAATGATAGAGTGTCAAC
Sequencing	
ES seq-ing 001	TCACTCAAAGGCGGTAA
ES seq-ing 002	TGTCGGGTCATGTGAGCAA
ES seq-ing 002 FW	ATGGCTCATAACACCCCTTGT
NGS_Forward primer	TTCTGCGCGTAATCTGCTGC
NGS_Reverse primer	GGCCTAACTACGGCTACACTAG
ES DEEP SEQ PET ini FW	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNTGACCATTCTGCGCGTAATCTGCTGC
ES DEEP SEQ PET vai FW	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNACAGTGTTCTGCGCGTAATCTGCTGC
ES DEEP SEQ PET RV	CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTGGCCTAACTACGGCTACACTAG
Digital PCR	

SC_dPCR_Chl_FW	AATAAAGGCCGGATAAAACTTG
SC_dPCR_Chl_RV	CTGGATATACCACCGTTGATAT
SC_dPCR_Chl_probe	/56-FAM/AATATCCAG/ZEN/CTGAACGGTCTGG/3IABKFQ/
SC_dPCR_ter_FW	AATAACATTCATTGGGTTGGTC
SC_dPCR_ter_RV	GCATGGTTAATCACGATGTAAT
SC_dPCR_ter_probe	/5HEX/AATAGCTAC/ZEN/CTCATCCGCGAAG/3IABKFQ/

Supplementary Table 1: Primers used in this work. All primers are shown in 5'→3' orientation. Chemical modifications for the primers used in digital PCR were as follows: /56-FAM/ - fluorescein; /ZEN/ - ZEN$^{\text{TM}}$ quencher; /3IABKFQ/ - Iowa Black® FQ; /5HEX/ - Hexachlorofluorescein.

Plasmid combination	# events
Intercompatibility experiments	
D4_1 (all)	2754
D4_1 (CM only)	43973
D4_1 (no ATB)	9029
G6 (all)	6736
G6 (CM only)	10225
G6 (no ATB)	3597
G4 (all)	2417
G4 (CM only)	9626
G4 (no ATB)	3046
Pairwise_intercompatibility experiments	
D4_2 + colE1 (CM)	8804
D4_2 + colE1 (no ATB)	6896
D4_1 + colE1 (no ATB)	1520
colE1 + G4 (no ATB)	8195
colE1 + G6 (no ATB)	5304
D4_1 + D4_2 (CM only)	1903
D4_1 + D4_2 (no ATB)	6851
D4_2 + G6 (CM only)	2625
D4_2 + G6 (no ATB)	9647
D4_2 + G4 (CM only)	1399
D4_2 + G4 (no ATB)	2428

Supplementary Table 2: Number of events post single-cell gating used in the analysis of plasmid populations. Naming of the experiments refers to the origins from each plasmid as described in the main text. Compatibility experiments carried out in the presence of kanamycin, chloramphenicol and ampicillin are shown as (all). Where only chloramphenicol was used in the experiment, samples are shown as (CM only). Experiments carried out in the absence of any antibiotic are shown as (no ATB).



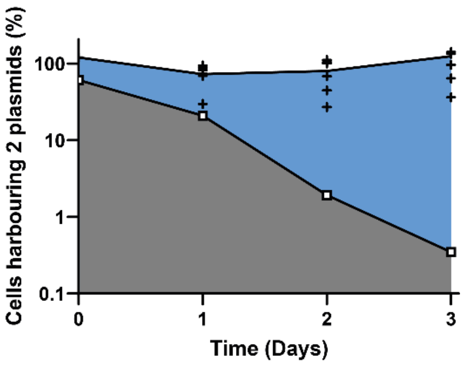
Variant ID	Hairpin 1	Hairpin 2	Hairpin 3	Frequency %(obs)
Library #1	CCA-CCGCNNNNAGCGG	AGAGCNNNNAACTCT	GGCTKNNNNNKAGCGCAGATACC-AAATACTG	**NA**
colE1-pET-29a(wt)	CCA-CCGCTACCAGCGG	AGAGCTACCAACTCT	GGCTTCAGCAGAGCGCAGATACC-AAATACTG	**NA**
26402_4709	CCA-CCGCCGAGAGCGG	AGAGCTAACAACTCT	GGCTTGCGCAGAGCGCAGATACC-AAATA-TG	4.35(1008)
21025_2610	CCA-CCGCTAAAAGCGG	AGAGCAAATAACTCT	GGCTTCAGCGGAGCGCAGATACC-AAATACTG	4.01(929)
16399_4158	CCA-CCGCATGGAGCGG	AGAGCTA-AGACTCT	GGCTGAAGCAGAGCGCAGATACC-AAATACTG	3.27(759)
16589_1792	CCA-CCGCCTTAAGCGG	AGAGCCTATAACTCT	GGCTTAAAACGAGCGCAGATAC--AAATACTG	2.57(596)
15324_1747	CCA-CCGCTTAAAGCGG	AGAGCAACTAACTCT	GGCTTCGCCAGAGCGCAGATAC--AAATACTG	2.28(529)
12414_2774	CCA-CCGCATAAAGCGG	AGAGCCATGAACTCT	GGCTGGTGCAGAGCGCAGATACC-AAATACTG	2.23(517)
21687_3318	CCA-CCGCCGCTAGCGG	AGAGCCCGGAACTCT	GGCTGCGGTTGAGCGCAGATAC--AAATACTG	1.86(431)
11760_2950	CCACCCGCCCAAAGCGG	AGAGCAAGTAACTCT	GGCTTCGGCCGAGCGCAGATACCCAAATACTG	1.82(423)
11167_2872	CCA-CCGCATGAAGCGG	AGAGCGGAAAACTCT	GGCTTGGCAATAGCGCAGATAC--AAATACTG	1.59(368)
9190_2197	CCA-CCGCCATAAGCGG	AGAGCCGATAACTCT	GGCTTTAGTCGAGCGCAGATA---AAATACTG	1.57(365)
20547_3194	CCA-CCGCCTCGAGCGG	AGAGCAATAAACTCT	GGCTTCAGTAGAGCGCAGATACC-AAATACTG	1.55(360)
8518_2431	CCA-CCGCTCTAAGCGG	AGAGCAAGCAACTCT	GGCTGGCGCAGAGCGCAGATACC-AAATACTG	1.55(359)
11988_8353	CCA-CCGCGCGTAGCGG	AGAGCATGAAACTCT	GGCTGAAAGAGAGCGCAGATAC--AAATACTG	1.51(351)
9429_2749	CCA-CCGCAGATAGCGG	AGAGCGAACAACTCT	GGCTTCTGCAGAGCGCAGATACC-AAATACTG	1.41(328)
20374_1778	CCA-CCGCCTTCAGCGG	AGAGCAGCAAACTCT	GGCTGAGGCAGAGCGCAGATACC-AAATACTG	1.41(327)
14057_2590	CCA-CCGCTAACAGCGG	AGA--TGCAAACTCT	GGCTTACCCAGAGCGCAGA-ACC-AAATACTG	1.40(324)
10100_5798	CCA-CCGCCACGAGCGG	AGAGCCAAGAACTCT	GGCTGTGGCAGAGCGCAGATACC-AAATACTG	1.35(313)
18614_6867	CCA-CCGCCACTAGCGG	AGAGCCCCAAACTCT	GGCTTCAGCAGAGCGCAGATACC-AAATACTG	1.34(310)
18810_2137	CCA-CCGCCACAAGCGG	AGAGCCCATAACTCT	GGCTGGGGCAGAGCGCAGATACC-AAATACTG	1.32(306)

Alpha	CCA-CCGCCACGAGCGG	AGAGCCAAGAACTCT	GGCTGTGGCAGAGCGCAGATACC-AAATACTG	**Bulk selection**
D4.I	CCA-CCGCCTAGAGCGG	AGAGCGACAAACTCT	GGCTTCTGCAGAGCGCAGATACC-AAATACTG	**Circuit screen**
D4.II	CCA-CCGCTTTGA-CGG	AGAGCGGCTAACTCT	GGCTGACGCAGAGCGCAGATACC-AAATACTG	**Circuit screen**
F4.I	CCA-CCGCGCGTAGCGG	AGAGCAGAGAACTCT	GGCTGATGCAGAGCGCAGATACC-AAATACTG	**Circuit screen**
G4.I	CCA-CCGTGTCGATCGG	AGAGCACCTAACTCT	GGCTTTGGTGGAGCGCAGATACC-AAATACTG	**Circuit screen**
G6.I	CCA-CCGCGGTGAGCGG	AGAGCGGTAAACTCT	GGGGCTGGTGGAGCGCAGATACC-AAATACTG	**Circuit screen**

Supplementary Figure 1: Viable colE1 origins identified by NGS and screening. A. NGS analysis of viable colE1 origins isolated from transformation of library #1. Mutations away from the wild-type sequence introduced by the library are shown in blue, mutations arising from selection are shown in red. Frequency of isolated origins is shown with the individual number of observations in brackets. The ID (automatically generated in sequencing) of one of the unique sequences is picked (arbitrarily) to name the group. NA – not applicable. **B.** Engineered colE1 origins described in this work.

Pipeline step	Sequences output
Total read number	31144
Quality trimming	31144 (100%)
Filtering by 5’ sequence	29916 (96%)
Filtering by 5’ sequence #2	29151 (94%)
Filtering by 3’ sequence	27175 (87%)
Filtering by 3’ sequence #2	23183* (74%)
Unique sequences	1903

Supplementary Table 3: Analysis by next generation sequencing of recovered viable origins. Total read number obtained and the impact of the analysis pipeline are shown. *Number of sequences used in downstream analysis.



Supplementary Figure 2: Compatibility selection in liquid culture. *E. coli* harbouring pSB1C3 (colE1 origin) and transformed with pET29 containing its wild-type (colE1; white squares) or a library of viable origins (black crosses) were serially passaged, with samples plated in the absence of antibiotics (to determine total CFU) or in the presence of both antibiotics (to determine CFU still harbouring both plasmids). As expected, under the growth conditions, the wild type colE1 origin is rapidly lost from the population.

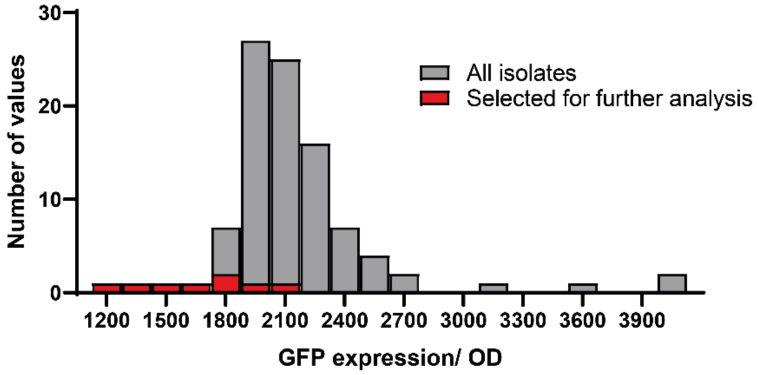
A

Variant ID	Hairpin 1	Hairpin 2	Hairpin 3	Frequency %(obs)
colE1_pET-29a(wt)	CCACCGCTACCAGCGG	AGAGCTACCAACTCT	GGCTTCAGCAGAGCGCAGATACCAAATACTG	**NA**
19921_1932	CCACCGCTACCAGCGG	AGAGCTACCAACTCT	GGCTTCAGCAGAGCGCAGATACCAAATACTG	(13432)
24147_3005 (alpha)	CCACCGCCACGAGCGG	AGAGCCAAGAACTCT	GGCTGTGGCAGAGCGCAGATACCAAATACTG	11.22(1433)
16596_2335	CCACCGCACCGAGCGG	AGAGCCGCCAACTCT	GGTTGACTAAGAGCGCAGATACCAAATACTG	4.01(512)
15490_2347	CCACCGCCATAAGCGG	AGAGCCGCCAACTCT	GGCTGGCAGAGAGCGCAGATACCAAATACTG	3.63(464)
23482_1919	CCACCGCAGAGAGCGG	AGAGCCCAAAACTCT	GGCTTCGGCAGAGCGCAGATACCAAATACTG	3.58(457)
11945_7277	CCACCGCACCTAGCGG	AGAGCCATCAACTCT	GGCTGACGCAGAGCGCAGATACCAAATACTG	3.52(450)
21796_3950	CCACCGCACATAGCGG	AGAGCTTTCAACTCT	GGCTTGAGCAGAGCGCAGATACCAAATACTG	3.21(410)
6435_3326	CCACCGCCACTAGCGG	AGAGCCCCAAACTCT	GGCTTCAGCAGAGCGCAGATACCAAATACTG	2.72(347)
13190_7993	CCACCGCAGAGAGCGG	AGAGCCCTCAACTCT	GGCTGTTGCAGAGCGCAGATACCAAATACTG	2.51(321)
13128_4999	CCACCGCCCACAGCGG	AGAGCCAGCAACTCT	GGCTGAGTAAGAGCGCAGA-ACCAAATACTG	2.47(315)
13613_5267	CCACCGCCACGAGCGG	AGAGCTACAAACTCT	GGCTTCGACAGAGCGCAGA-ACCAAATACTG	2.43(310)
16898_3487	CCACCGCCATGAGCGG	AGAGCCCGCAACTCT	GGCTTGACCTGAGCGCAGATA-CAAATACTG	1.97(252)
25074_2902	CCACCGCAGACAGCGG	AGAGCCTCTAACTCT	GGCTGACGCTGAGCGCAGA-ACCAAATACTG	1.96(251)
21215_6358	CCACCGCACCAAGCGG	AGAGCGCTTAACTCT	GGCTTACGCAGAGCGCAGATACCAAATACTG	1.96(250)
12850_3238	CCACCGCCCTAAGCGG	--AGCCATTAACTCT	GGCTTATGCAGAGCGCAGATACCAAATACTG	1.78(227)
25300_8619	CCACCGCAGATAGCGG	AGAGCCAACAACTCT	GGCTTCCGCAGAGCGCAGATACCAAATACTG	1.76(225)
14580_2678	CCACCGCCACCAGCGG	AGAGCTATTAACTCT	GGCTTCGGCAGAGCGCAGATACCAAATACTG	1.73(221)

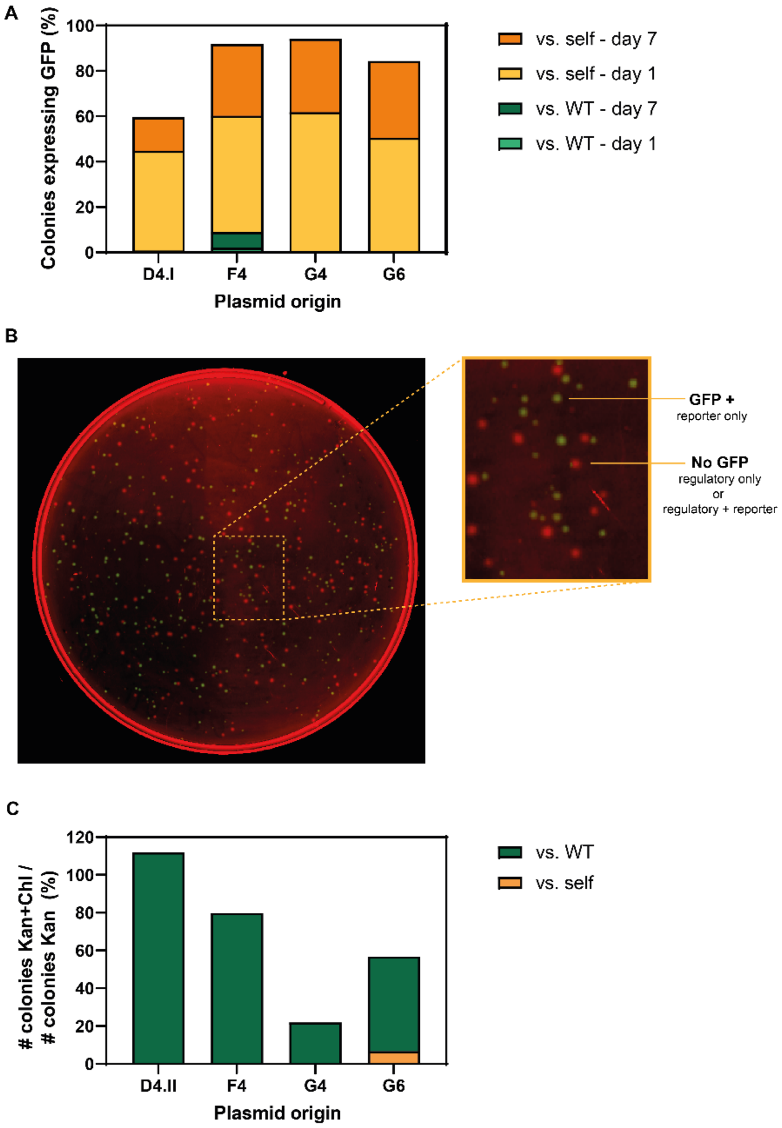
Supplementary Figure 3: NGS analysis after large-scale selection for plasmid compatibility. NGS analysis of colE1 origins isolated after selection of viable colE1 variants co-transformed with wild-type colE1. Mutations away from the wild-type sequence introduced by the library are shown in blue, mutations arising from selection are shown in red. Frequency of isolated origins is shown with the individual number of observations in brackets. Wild-type colE1 sequences were identified in the experiment (a limitation of the approach used to prepare plasmid DNA for NGS) and are excluded from the analysis – the number of observations is still given. The ID (automatically generated in sequencing) of one the unique sequences is picked (arbitrarily) to name the group. NA – not applicable.

Pipeline step	Sequences output
Total read number	28438
Quality trimming	28438 (100%)
Filtering by 5’ sequence	27876 (98%)
Filtering by 5’ sequence #2	27708 (97%)
Filtering by 3’ sequence	26777 (94%)
Filtering by 3’ sequence #2	26206* (92%)
Unique sequences	1185

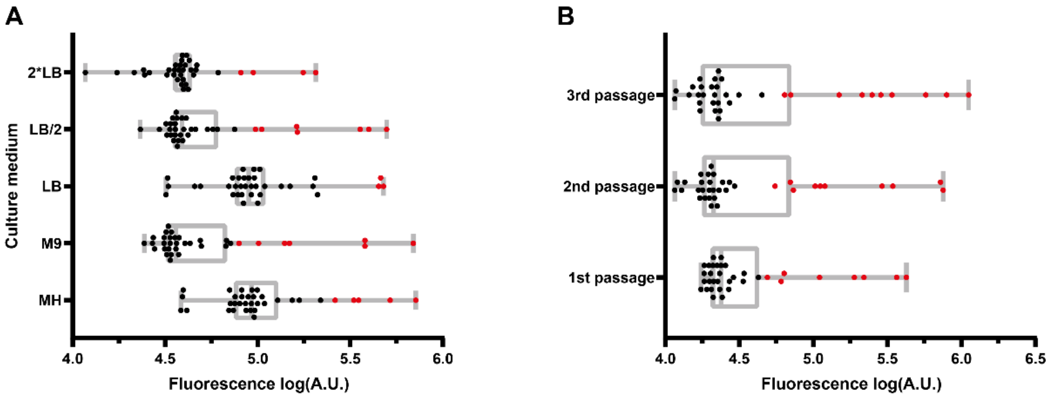
Supplementary Table 4: Analysis by next generation sequencing of recovered compatible origins. Total read number obtained and the impact of the analysis pipeline are shown. *Number of sequences used in downstream analysis.



Supplementary Figure 4: High-throughput screening assay for the selection of colE1-compatible origins of replication. Histogram representation of the data shown in Figure 3C, highlighting the fluorescence values of the variants selected for further study.



Supplementary Figure 5: Characterisation of selected colE1 origin variants for their compatibility with colE1. A. Serial cultures of *E. coli* cells co-transformed with reporter (harbouring one of D4.I, F4, G4, G6 and wild-type origins of replication) and regulatory (harbouring D4.I, F4, G4 or G6 origins) were used to test the compatibility of the selected variants against wild-type and to confirm their self-incompatibility. The percentage of cells expressing GFP was calculated by diluting a culture aliquot (after 1 or 7 days of passaging) and plating in LB agar supplemented with chloramphenicol (to retain reporter plasmid). Bar graphs are overlaid with compatibility to wild-type shown in green (light or dark depending on passage number) and self-incompatibility shown in orange (light or dark depending on passage number). **B.** Example of transformation plate used to calculate values in **A.** Here, D4.I was used in both regulatory and reporter plasmids and the results show the distribution of plasmids after 7 days of passaging. Fluorescent images from GFP (green) and control channels (red) are overlaid and CFU counted. **C.** Complementary experiment where after passaging in the absence of antibiotic selection, cultures are plated in media supplemented with kanamycin (regulatory plasmid antibiotic marker) or with both antibiotics to monitor plasmid loss. Bar graphs are overlaid with compatibility to wild-type shown in green and self-incompatibility shown in orange.



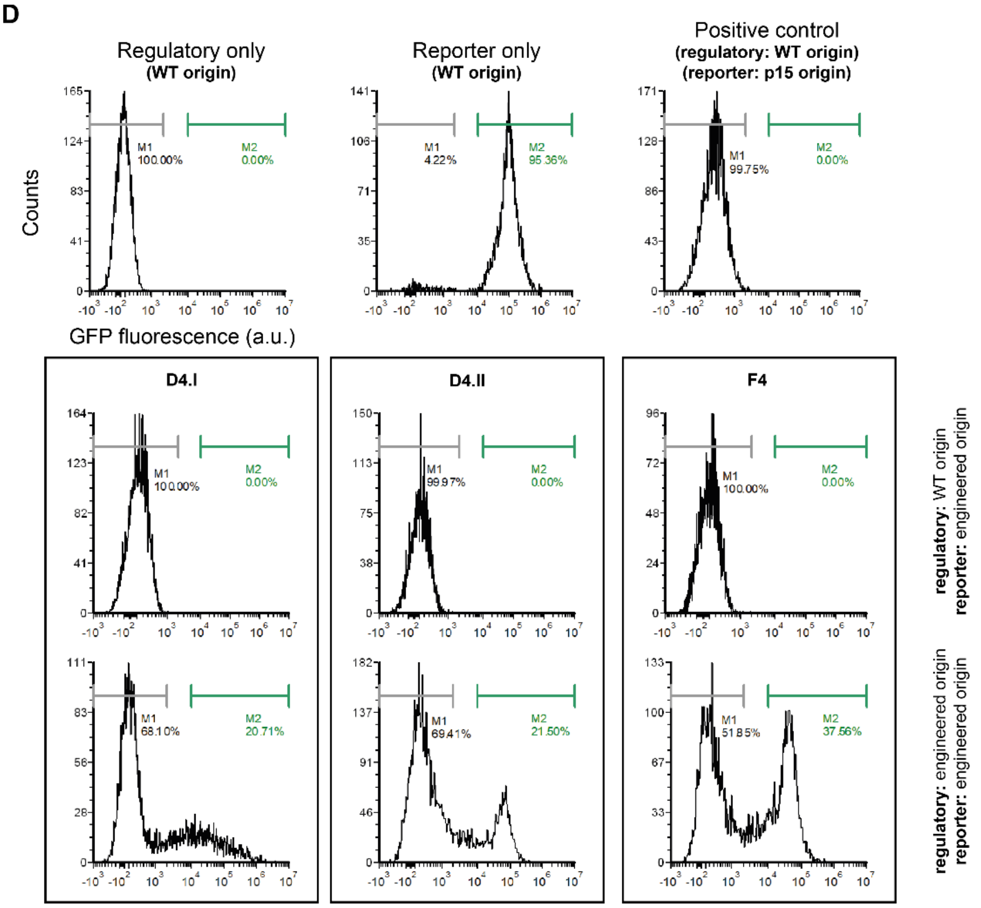
C

1st dilution							
M9		pGFP(TetA)					
		WT	D4 I	G6	D4 II	F4	G4
pTetR	WT		28838	27170	24209	23776	23285
	D4 I	19704	34078	25503	18343	20212	20976
	G6	23319	220142	365037	22609	20490	22387
	D4 II	21133	42589	21106	60662	18349	18293
	F4	19862	26849	21184	19061	63579	17417
	G4	33635	110283	425005	23830	48928	189417

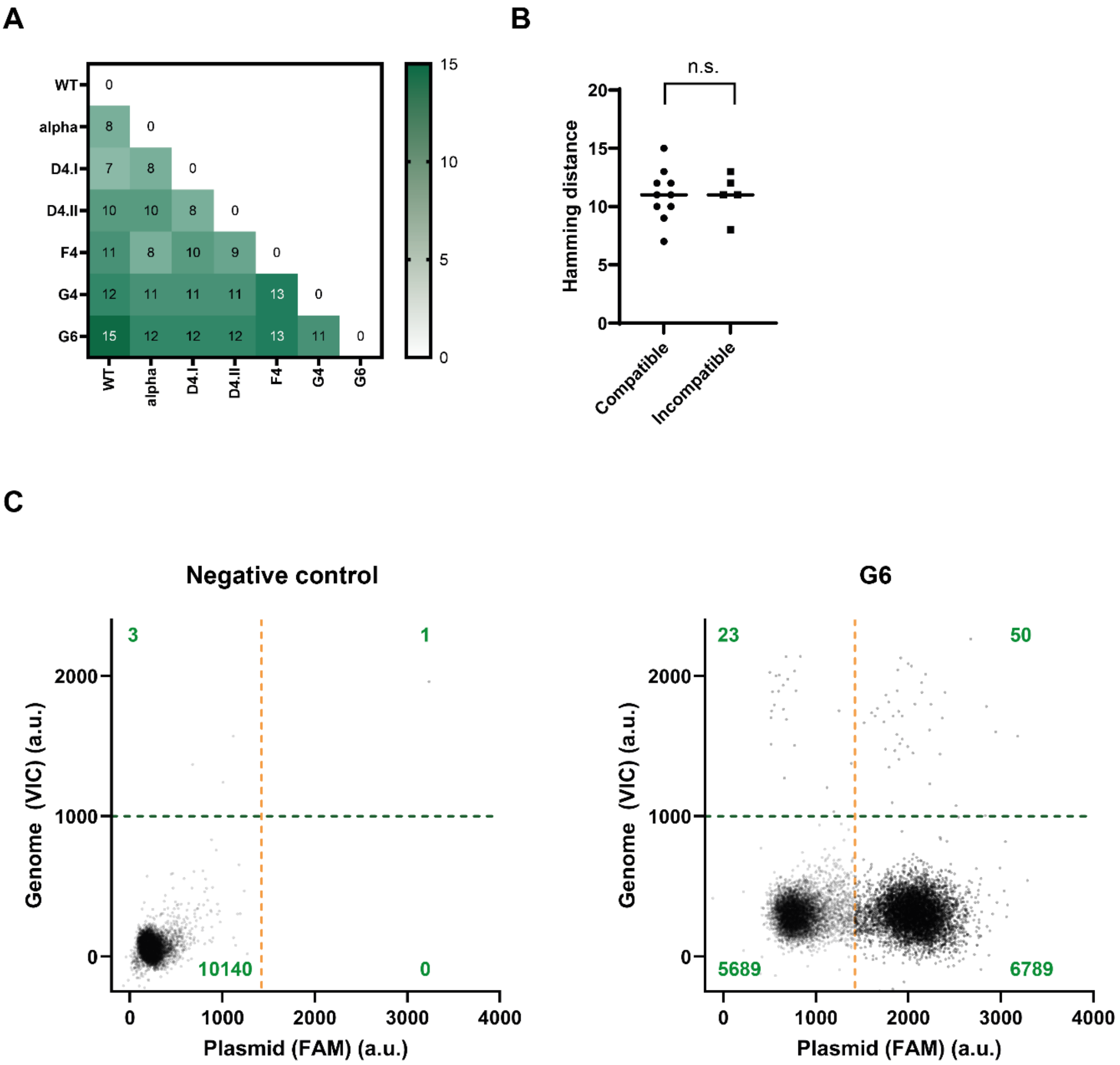
pGFP(TetA) positive control	387180
pTetR negative control	19953

3rd dilution							
M9		pGFP(TetA)					
		WT	D4 I	G6	D4 II	F4	G4
pTetR	WT		21699	21105	22897	15320	16763
	D4 I	20591	574962	17190	17078	17176	18047
	G6	27180	213569	796289	22905	14470	21593
	D4 II	22785	64041	11803	339829	21765	23394
	F4	22291	45029	11608	24051	249330	17521
	G4	25794	70443	1119908	31474	149623	286265

pGFP(TetA) positive control	738296
pTetR negative control	22328

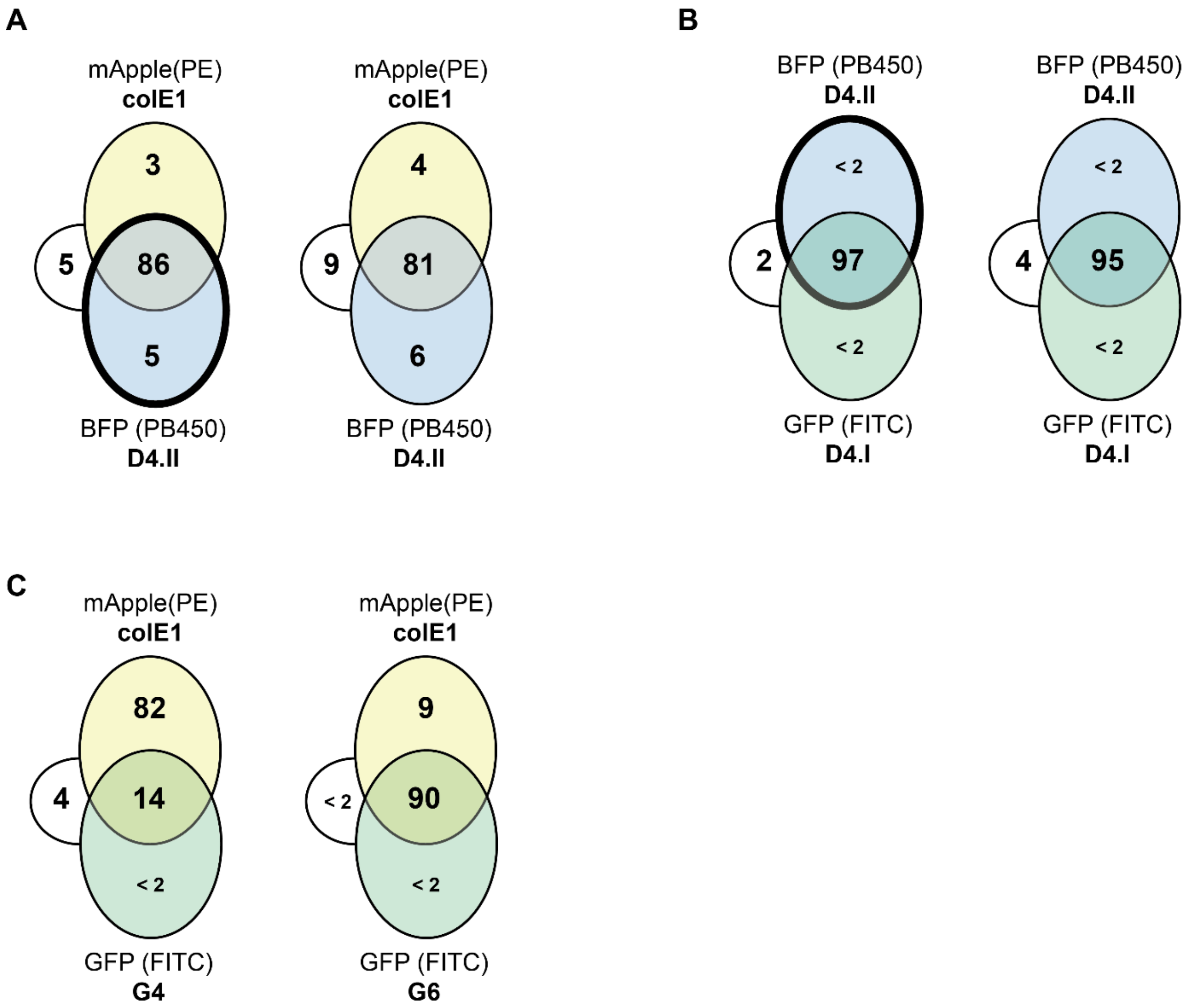


Supplementary Figure 6: Impact of culture medium on plasmid compatibility and characterization of cross-compatibility. A. Box plot showing the distribution of normalized fluorescence for cross-compatibility assays carried out in different culture media (single passage). Outliers (2% cut-off in ROUT analysis), that is significantly expressing GFP, are shown in red. **B.** Box plot showing the impact of passaging in cross-compatibility assays in M9 media. Outliers (2% cut-off in ROUT analysis), that is significantly expressing GFP, are shown in red. **C.** Cross-compatibility results (normalized fluorescence values) obtained in M9 after one or two passages. Outliers identified in **B.** are shown in green. The third passage is shown in Figure 4A. **D.** Flow cytometry analysis of cross-compatibility assays showing controls and selected replication origins, their compatibility to wild-type colE1 origins and their self-incompatibility. Markers show ranges used to quantify non-fluorescence (grey) and fluorescent (green) fractions of the populations. Each experiment included at least 6200 events post single-cell gating.

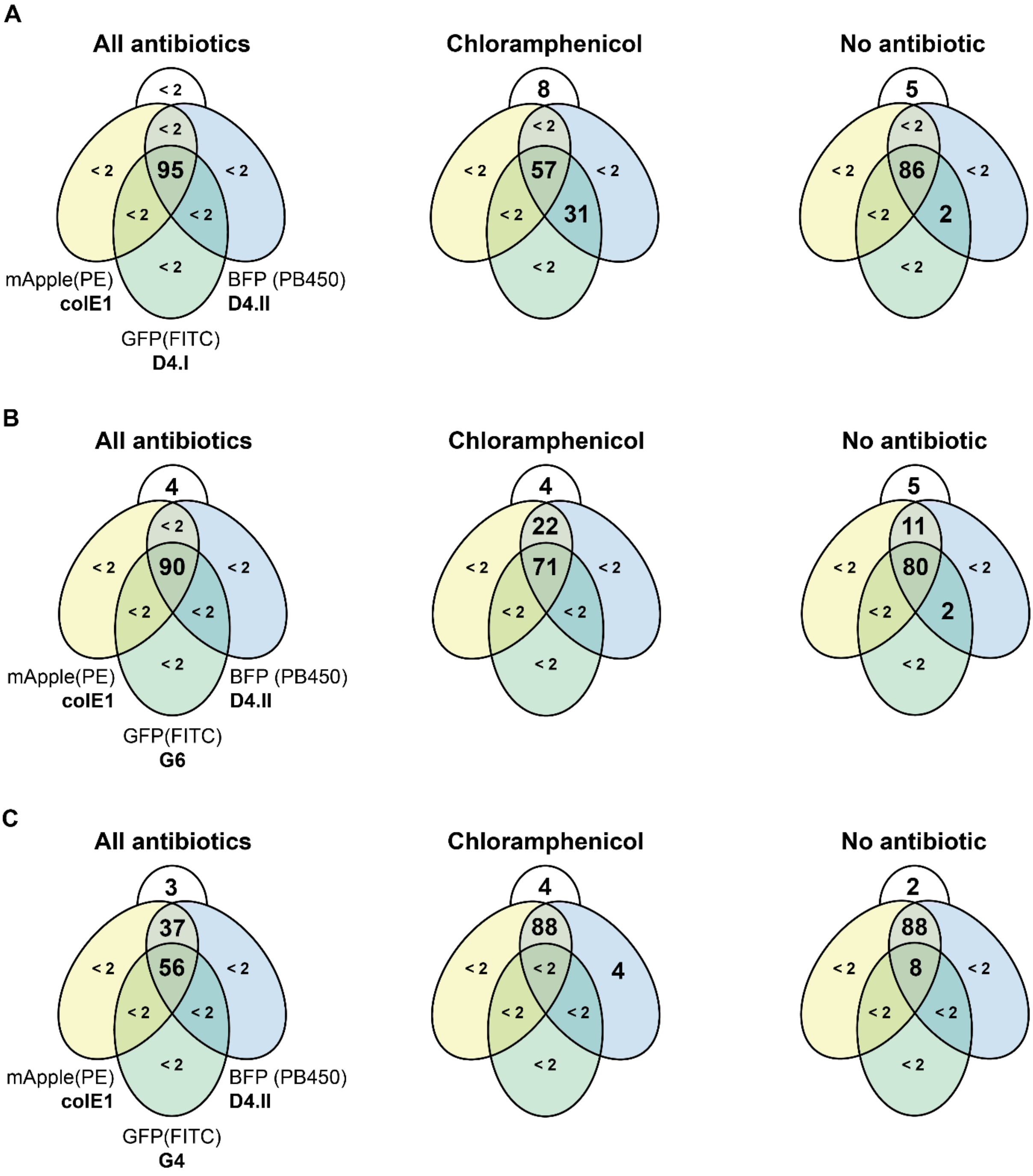


	WT	alpha	D4.I	D4.II	F4	G4	G6
WT	0						
alpha	8	0					
D4.I	7	8	0				
D4.II	10	10	8	0			
F4	11	8	10	9	0		
G4	12	11	11	11	13	0	
G6	15	12	12	12	13	11	0

Supplementary Figure 7: Sequence analysis of engineered colE1 origins and quantification of plasmid copy number per cell. A. Hamming distance (number of substitutions between 2 sequences) between the engineered origins of replication. **B.** Hamming distance distribution between compatible and incompatible colE1 origins (using data presented in Figure 4A). A Kolmogorov-Smirnov test was used to compare the two Hamming distance distributions but no significant different was observed. **C.** Examples of digital PCR results to show negative control (no template) and results obtained for G6 engineered origin. The quadrants are determined automatically by the analysis program and the number of observations in each quadrant are shown in green.



Supplementary Figure 8: Pairwise compatibility between origins in plasmids expressing fluorescent proteins. Summary of flow cytometry analysis of cultures post-serial passaging in M9 used to investigate plasmid retention and plasmid compatibility. Origins and fluorescent protein encoded are shown around the edges of the Venn diagram: D4.II origin in mTagBFP2-pBAD (blue), colE1 origin in mApple-pBAD (yellow) and other origins in GFP-pBAD (green). Thick borders show experiments where chloramphenicol was used to ensure D4.II plasmid retention. **A.** D4.II and colE1 origins. **B.** D4.I and D4.II origins. Both show that chloramphenicol selection has little impact on the retention of the plasmids. **C.** colE1 origins and G4 or G6. Under the culture conditions used for this experiment, the G4 origin is lost from the population (in alignment with what was seen in SI Fig 5C, but different from what was observed in the high-throughput assay (Figure 4A). These experiments were also used as controls for the 3-way intercompatibility assays.



Supplementary Figure 9: Plasmid intercompatibility assays. Summary of flow cytometry analysis of cultures post-serial passaging in M9 used to investigate plasmid retention and plasmid compatibility. Cells co-transformed with three plasmids harbouring different plasmid origin combinations were serially passaged in M9 before being analysed by flow cytometry to determine which plasmids had been retained in culture. Plasmid origins and fluorescent proteins are shown for each combination around the Venn diagram. BFP is shown in blue, mApple in yellow and GFP in green. Cultures were maintained with all antibiotics (ampicillin, chloramphenicol and kanamycin), or with only chloramphenicol, or without any added antibiotics. **A.** Origins D4.I, D4.II and wild-type colE1. Passaging of the culture in the presence of chloramphenicol results in significant wild-type colE1 loss. **B.** Origins G6, D4.II and colE1. Plasmid harbouring G6 origin is preferentially lost from culture but at slow rates, ensuring that most of the population retains all 3 plasmids. **C.** Origins G4, D4.II and wild-type colE1. In contrast to the pairwise assays, plasmids with the G4 origin were rapidly lost from the population, even in the presence of all three antibiotics, suggesting that it may not as stable as other origins or that its low copy number puts it in a significant disadvantage during replication.

Supplementary notes:

Polymerase Chain Reaction. PCR was used to generate the biological constructs for this work. Unless stated otherwise, all reactions were carried out in 50 µL with the following reaction components: 1X Q5 reaction buffer, 0.5 µM of each primer, 200 µM dNTPs, 0.2 ng/µL of template, 0.02 U/µl Q5 enzyme (New England Biolabs), and deionized sterile water to complete the reaction volume. The reaction conditions typically consisted of an initial denaturation at 95°C for 30 seconds, followed by 30 – 32 cycles of 95°C for 20 seconds, 50 - 72°C for 30 seconds, 72°C for 30 seconds/kb of the target DNA product. All reactions included final 72°C extension for 5 minutes.

Raw data. All data and analyses generated in this project are publicly available at https://github.com/PinheiroLab/Engineered_colE1_origins.