

Supplemental material**Supplemental method:** Chromatin Immunoprecipitation.

After formaldehyde treatment and cell lysis and sonication as described in the methods section, DNA was further reduced to 200-400 bp fragment sizes by addition of 25 μ L micrococcal nuclease digestion solution (15 mM Tris-HCl pH 8.0, 1 mM CaCl_2 , 60 mM KCl, 15 mM NaCl, 300 mM sucrose, 0.5 mM DTT) followed by 5 μ L of 2000 U/ μ L micrococcal nuclease (New England Biolabs). Following incubation at 37°C for 20 min, each reaction was stopped by addition of 125 μ L of 0.5 M EDTA solution. Cellular debris was removed by centrifugation. A 50- μ L lysate aliquot was used for each immunoprecipitation. To this aliquot was added 200 μ L IP buffer and 5 μ g specific antibody [(monoclonal antibodies to RNA polymerase α subunit or σ^{70} subunit (Neoclone)]. Immunoprecipitation samples were incubated overnight at 4° C with slow rotation. 50 μ L of UltraLink protein A/G beads (Thermo Scientific) were then added to each sample with slow rotation at room temperature for 2 h. Beads were collected by centrifugation in Spin-X 0.22 μ m CA spin filters (Costar). Samples were washed once with IP buffer, once with LiCl wash buffer (250 mM LiCl, 100 mM Tris pH 8.0, 2% Triton X-100), twice with 600 mM NaCl wash buffer (100 mM Tris-HCl 8.0, 600 mM NaCl, 2% Triton X-100), twice with 300 mM NaCl wash buffer (100 mM Tris-HCl 8.0, 300 mM NaCl, 2% Triton X-100) and twice with a solution of 10 mM Tris-HCl (pH 8) containing 1 mM EDTA. DNA/protein complexes were collected by elution in 200 μ L of buffer containing 50 mM Tris-HCl 8.0, 10 mM EDTA, 1% SDS at 65°C for 30 min. RNaseA (10 μ L of 10 mg/mL stock) and proteinase K (10 μ L of 10 mg/mL stock) were added to each sample. DNA-protein crosslinks were reversed by overnight incubation at

65°C. DNA was then purified using a QIAquick PCR clean up kit (Qiagen) with elution in a final volume of 200 µL of water. Real-time PCR was performed in triplicate using a Bio-Rad iCycler and a PerfeCTa™ SYBR Green Fast Mix™ for iQ real-time PCR kit (Quanta Biosciences) as specified by the manufacturer. PCR primers specific for the *lac* promoter region in the F128 episome (5'-TATCACGAG₂C₃T₃CGTC and 5'-CGTA₂TCATG₂TCATAGCTGT₃C) were used to determine DNA fragment enrichment. PCR cycling was: 95°C (30 s); 40 cycles of 60°C (30 s), 95°C (1 min), 55°C (2 min). Denaturation curves were generated in the instrument to confirm amplification of a single PCR product. Analysis was based on the threshold cycle (C_t). Differences in starting lysate amounts were corrected using values calculated from 10% input controls and fold enrichment used a promoter-less control for normalization. All ChIP assays were repeated three times.

Table S1**Downstream O2/Osym**

spacing(a)	plasmid	strain
69.5	pJ1911	BL1072
68.5	pJ1912	BL1074
67.5	pJ1910	BL1068
62.5	pJ1888	BL1046
60.5	pJ1889	BL1048
58.5	pJ1890	BL1050
56.5	pJ1852	BL990
54.5	pJ1838	BL992
52.5	pJ1839	BL1024
50.5	pJ1840	BL994
48.5	pJ1853	BL996
46.5	pJ1841	BL998
44.5	pJ1842	BL1000
42.5	pJ1843	BL1002
41.5	pJ1893	BL1056
40.5	pJ1854	BL1004
39.5	pJ1894	BL1058
38.5	pJ1844	BL1006
37.5	pJ1895	BL1060
36.5	pJ1845	BL1008
35.5	pJ1896	BL1062
34.5	pJ1846	BL1010
33.5	pJ1897	BL1064
32.5	pJ1847	BL1012
30.5	pJ1855	BL1014
28.5	pJ1848	BL1016
26.5	pJ1837	BL1018
25.5	pJ1898	BL1066
24.5	pJ1861	BL1026
22.5	pJ1862	BL1028
20.5	pJ1863	BL1030

Downstream Osym only

spacing (b)	plasmid	strain
67.5	pJ1935	BL1087
66.5	pJ1908	BL1070
62.5	pJ1934	BL1088
56.5	pJ1849	BL1044
50.5	pJ1779	BL968
44.5	pJ1932	BL1081
42.5	pJ1933	BL1083
41.5	pJ1936	BL1089

(a) Indicates spacing in bp from center of O2 to center of Osym.

(b) Indicates spacing in bp from center of dead O2 to center of functional Osym.

Table S2

upstream	E-IPTG average(*)	sd(*)	E+IPTG average(*)	sd(*)
Osym-O2				
49.5				
50.5				
51.5				
52.5				
53.5				
54.5				
55.5				
57.5				
59.5				
62.5				
63.5	16.113	6.525	905.081	130.881
64.5	6.118	1.026	593.962	85.541
65.5	6.776	0.810	671.263	144.889
66.5	5.911	0.577	367.261	84.026
67.5	4.344	0.641	155.750	37.047
68.5				
69.5				
70.5	16.895	2.489	522.824	74.554
71.5	17.092	3.022	553.266	72.633
72.5	19.596	4.240	568.988	124.858
73.5	32.972	8.475	734.275	86.648
74.5	24.414	7.842	635.396	97.210
75.5	12.227	1.621	660.628	67.778
76.5	6.179	1.054	414.782	65.594
77.5	4.586	0.579	326.426	62.478
78.5	4.385	0.452	222.558	46.877
79.5	5.395	0.574	236.466	43.836
80.5	8.299	1.528	345.038	53.205
81.5	10.145	1.603	396.226	72.239
82.5	12.257	2.145	441.869	65.910
83.5	21.979	3.040	476.839	62.281
84.5	31.112	7.855	464.427	55.665
85.5	16.982	3.687	456.611	29.975
86.5	8.355	1.820	421.753	73.024
87.5	5.304	0.476	286.891	51.174
88.5	7.154	1.058	168.242	24.018
89.5	4.620	0.776	157.051	25.593
90.5	5.787	1.207	180.356	29.883
O2 alone	262.716	40.240	798.207	85.917
Osym alone				
76.5	994.73	240.77	1390.9	226.89
77.5	1007.44	67.71	1144.58	59.89
78.5	1068.85	64.47	1203.13	121.06
79.5	1261.7	111.52	1248.18	63.19
80.5	1666.96	236.12	1395.66	94.86
81.5	1693.8	148.36	1521.66	97.32
82.5	1473.84	353.19	1476.34	315.78
83.5	1146.98	164.71	1062.63	124.26
84.5	846.56	19.74	1013.71	24.64
85.5	693.49	76.81	895.8	92.73
86.5	879.54	8.29	1066.6	11.07
87.5	913.81	73	1042.96	77.77
88.5	994.66	42.15	1024.98	79.56
no operators	1443.08	102.22	1466.13	87.27
downstream				
O2-Osym	E-IPTG average	sd	E+IPTG average	sd
20.5	0.685	0.051	3.403	0.168
22.5	0.513	0.067	6.751	0.325
24.5	0.364	0.042	3.000	0.104
25.5	0.959	0.160	9.906	0.453
26.5	8.847	1.218	238.402	35.571
28.5	4.620	0.975	80.371	6.575
30.5	6.433	0.675	108.796	9.751

(*) data reported by Becker et al. (2005).

32.5	8.534	0.493	146.640	5.251
33.5	6.565	1.514	110.609	13.409
34.5	20.456	2.573	313.103	37.220
35.5	17.288	4.536	135.868	12.903
36.5	14.636	1.124	211.811	22.888
37.5	18.332	5.117	210.619	44.670
38.5	54.550	7.107	714.237	52.016
39.5	23.438	5.740	117.959	9.208
40.5	13.194	1.017	225.591	51.014
41.5	11.603	2.080	131.024	51.883
42.5	49.320	3.405	391.025	47.550
44.5	7.418	1.594	204.004	52.505
46.5	8.175	1.852	446.183	136.948
48.5	10.149	0.482	154.433	5.493
50.5	15.724	3.431	221.279	23.833
52.5	6.490	1.687	121.910	32.253
54.5	5.793	0.508	284.004	38.006
56.5	0.341	0.046	23.328	2.309
58.5	0.569	0.063	40.383	3.076
60.5	10.055	2.499	457.038	121.886
62.5	21.044	7.326	270.475	34.750
67.5	0.41	0.109	27.731	2.028
68.5	1.425	0.375	48.981	4.366
69.5	0.74	0.165	36.042	2.958
O2 alone	95.225	15.535	188.651	43.866

Osym alone

41.5	15.6	1.63	76.86	6.9
42.5	54.64	14.1	189.69	20.73
44.5	48.9	5.61	191.75	17.21
50.5	5.92	0.37	29.91	1.49
56.5	1.98	0.26	20.81	1.29
62.5	3.69	0.34	9.85	1.4
66.5	25.98	1.79	196.08	5.31
67.5	0.82	0.13	7.34	0.87
no operators	64.60	2.94	61.60	5.14

LacI mutant	E-IPTG		E+IPTG	
	average	sd	average	sd
O2-Osym				
36.5	271.814	32.891	269.03	12.766
37.5	429.893	55.769	411.064	84.507
38.5	806.844	120.659	913.635	208.824
39.5	235.466	35.148	210.572	27.722
40.5	387.467	81.653	330.158	56.642
41.5	270.565	29.381	281.686	9.858
48.5	276.144	43.021	266.698	39.317
50.5	255.714	55.493	227.438	30.357
54.5	392.49	53.032	384.617	40.001
56.5	195.12	18.366	188.388	13.94
58.5	145.392	38.871	149.797	47.84
60.5	791.576	156.709	778.653	163.355
62.5	503.346	30.343	516.66	19.979
67.5	161.304	22.55	169.67	12.964
69.5	153.51	19.362	154.986	14.776
O2 alone	751.705	76.494	711.274	22.601
Osym alone				
42.5	301.319	28.342	260.077	18.897
44.5	413.643	70.533	388.05	50.58
50.5	45.27	7.897	39.863	7.274
56.5	30.915	5.509	25.964	5.789
62.5	9.495	0.731	7.717	0.495

Table S3

	promoter set-up	E-IPTG		E+IPTG		E+Ara		E+Ara+IPTG		ratio +AI/+A	
		average	sd	average	sd	average	sd	average	sd	average	sd
BL1076	Osym-T7-O1	0.42	0.05	4.80	1.16	1.79	0.83	30.18	7.45	19.70	9.30
BL1093	T7-O1	17.77	9.30	17.33	8.63	127.24	12.09	62.95	24.56	0.50	0.21
BL1095	T7-O1-Osym	13.61	6.51	13.08	5.41	94.17	10.01	54.76	20.29	0.59	0.24

Table S4

plasmid	strain	operators^a	insert (bp)^b
pJ1778	BL966	O2/Osym	0
pJ1811	BL974	O2/Osym	+20
pJ1812	BL976	O2/Osym	+40
pJ1779	BL968	-/Osym	0
pJ1813	BL978	-/Osym	+20
pJ1814	BL980	-/Osym	+40
pJ1780	BL970	O2/-	0
pJ1815	BL982	O2/-	+20
pJ1816	BL984	O2/-	+40
pJ1781	BL972	-/-	0
pJ1817	BL986	-/-	+20
pJ1818	BL988	-/-	+40

^a Indicates functional or dead operators in this study. In all cases, the operator center to center distance is 50.5 bp.

^b Indicates bp size of insert added between transcriptional start site and proximal operator (functional or dead).

Table S5

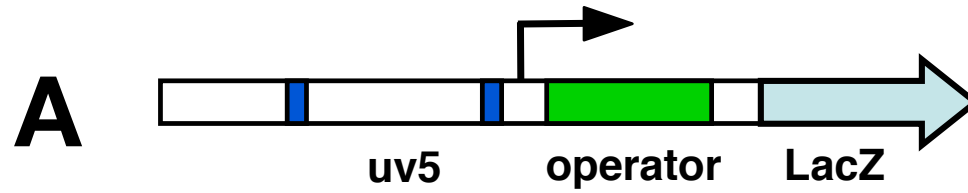
spacing (bp)^a	plasmid	strain	promoter^b	location^c	loop^d
O2 only	pJ992	BL546	UV5		
84.5	pJ967	BL596	UV5	upstream	twisted
77.5	pJ962	BL600	UV5	upstream	relaxed
62.5	pJ1888	BL1046	UV5	downstream	twisted
58.5	pJ1890	BL1050	UV5	downstream	relaxed
86.5	pJ1906	BL1076	T7	upstream	relaxed
58.5	pJ1940	BL1095	T7	downstream	relaxed
O1 only	pJ1938	BL1093	T7		

^a Indicates operator center to center distance in bp. Samples with only a single functional operator are indicated.

^b Constructs contain either a UV5 or T7 transcriptional promoter. The T7 promoters serve as a promoterless control since T7 RNA polymerase is not being expressed in these cells at the time of assay.

^c Location of the operators relative to the transcriptional start site.

^d Indicates the looping flexibility of constructs based on in vivo β -gal assay.



		plasmid	strain
Osym	AATTGTGAGC GCTCACAATT	pJ949	BL539
O1	AATTGTGAGCGGATAACAATT	pJ991	BL683
O2	AAaTGTGAGCGagTACAacc	pJ992	BL546
O3	ggcaGTGAGCGcAacgCAATT	pJ993	BL548

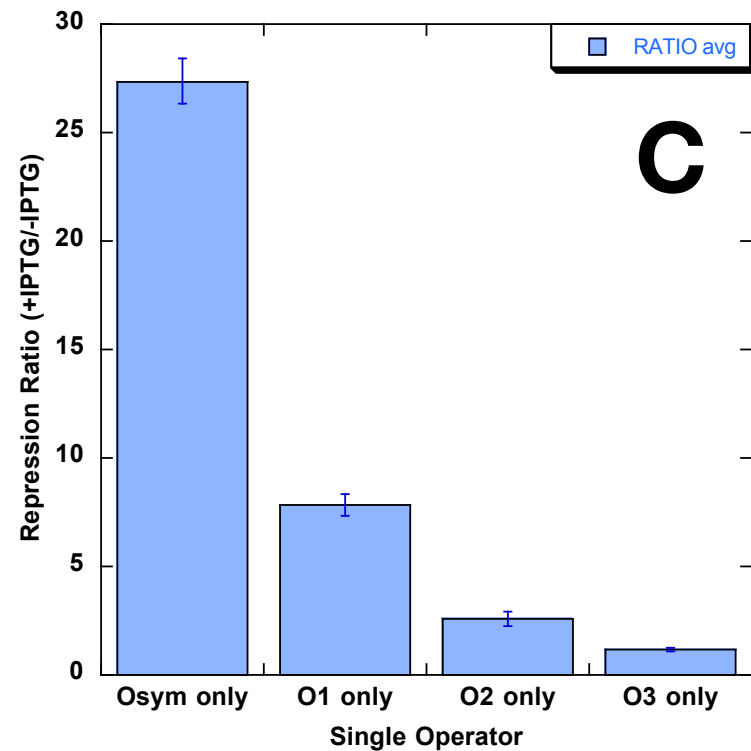
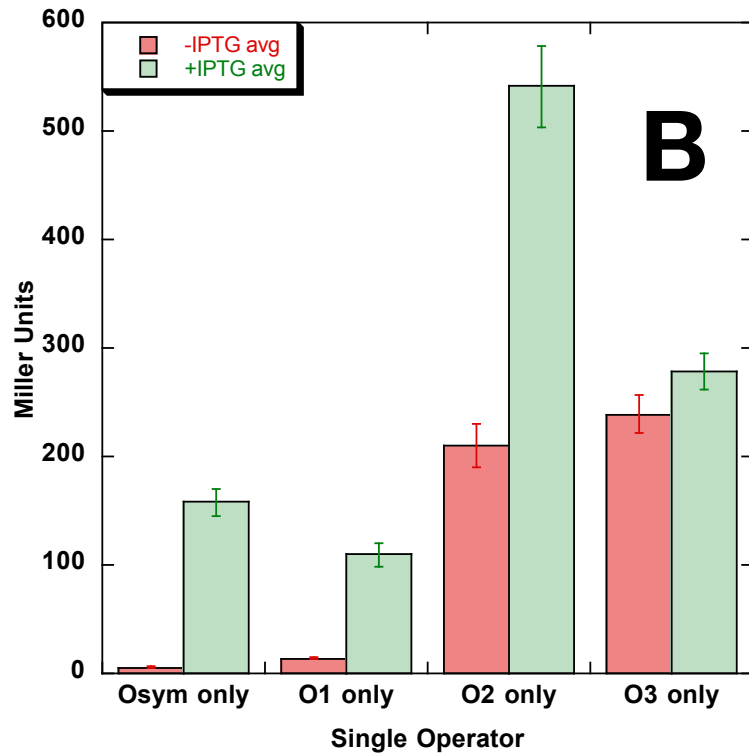
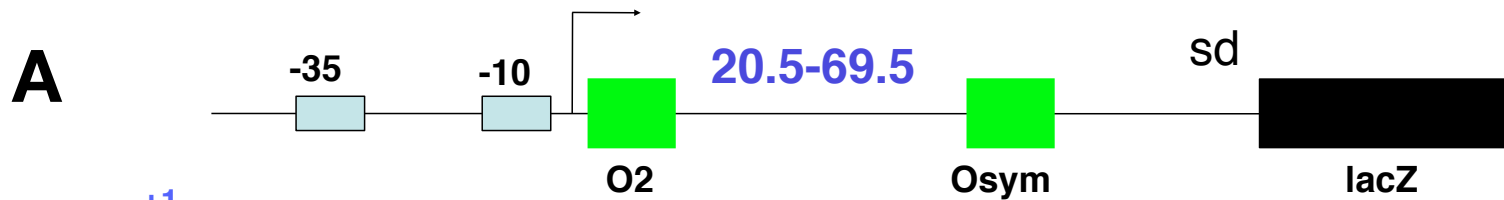


figure s1



B

+1 ●

	spacing	plasmid	strains
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C G C C G G A G C T C T G G T A C C C T G G A T C C a c g c t a G C A A T T G T G A G C G C T C A C A A T T	69.5	1911	1072/1073
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C G C C G G A G C T C T G G T A C C C T G G A T C C a c g c t a G C A A T T G T G A G C G C T C A C A A T T G	68.5	1912	1074/1075
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C G C C G G A G C T C T G G T A C C C T G G A T C C a c g c t a A A T T G T G A G C G C T C A C A A T T G C	67.5	1910	1068/1069
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C C C T G G A T C C a c g c t a G C G G C C A A T T G T G A G C G C T C A C A A T T G C	62.5	1888	1046/1047
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C C C T G G A T C C a c g c t a G C G G A A T T G T G A G C G C T C A C A A T T C C G C	60.5	1889	1048/1049
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C C C T G G A T C C a c g c t a G C A A T T G T G A G C G C T C A C A A T T G G C C G C	58.5	1890	1050/1051
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C C C T G G A T C C a c g c t a A A T T G T G A G C G C T C A C A A T T G C G G C C G C	56.5	1852	990/991
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C C C T G G A T C C a c g c t a A A T T G T G A G C G C T C A C A A T T t a G C G G C C G C	54.5	1838	992/993
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C C C T G G A T C C a c g c t a A A T T G T G A G C G C T C A C A A T T g c t a G C G G C C G C	52.5	1839	1024/1025
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GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C C C T G G A T C C a c g c t a A A T T G T G A G C G C T C A C A A T T C C a c g c t a G C G G C C G C	48.5	1853	996/997
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C C C T G G A A T T G T G A G C G C T C A C A A T T A T C C a c g c t a G C G G C C G C	46.5	1841	998/999
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C C C T A A T T G T G A G C G C T C A C A A T T G G A T C C a c g c t a G C G G C C G C	44.5	1842	1000/1001
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C C A A T T G T G A G C G C T C A C A A T T C T G G A T C C a c g c t a G C G G C C G C	42.5	1843	1002/1003
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A C A A T T G T G A G C G C T C A C A A T T C C T G G A T C C a c g c t a G C G G C C G C	41.5	1893	1056/1057
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A A A T T G T G A G C G C T C A C A A T T C C C T G G A T C C a c g c t a G C G G C C G C	40.5	1854	1004/1005
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G T A A T T G T G A G C G C T C A C A A T T A C C C T G G A T C C a c g c t a G C G G C C G C	39.5	1894	1058/1059
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G G A A T T G T G A G C G C T C A C A A T T T A C C C T G G A T C C a c g c t a G C G G C C G C	38.5	1844	1006/1007
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T G A A T T G T G A G C G C T C A C A A T T G T A C C C T G G A T C C a c g c t a G C G G C C G C	37.5	1895	1060/1061
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C T A A T T G T G A G C G C T C A C A A T T G G T A C C C T G G A T C C a c g c t a G C G G C C G C	36.5	1845	1008/1009
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C C A A T T G T G A G C G C T C A C A A T T T G G T A C C C T G G A T C C a c g c t a G C G G C C G C	35.5	1896	1062/1063
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T G C A A T T G T G A G C G C T C A C A A T T C T G G T A C C C T G G A T C C a c g c t a G C G G C C G C	34.5	1846	1010/1011
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GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A T A A T T G T G A G C G C T C A C A A T T G C C T G G T A C C C T G G A T C C a c g c t a G C G G C C G C	32.5	1847	1012/1013
GTCGACC A A A T G T G A G C G A G T A A C A A C C G A A T T C C T G C A A T T G T G A G C G C T C A C A A T T A T G C C T G G T A C C C T G G A T C C a c g c t a G C G G C C G C	30.5	1855	1014/1015
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GTCGACC A A A T G T G A G C G A G T A A C A A C C A A T T G T G A G C G C T C A C A A T T G A A T T C C T G C A T G C C T G G T A C C C T G G A T C C a c g c t a G C G G C C G C	20.5	1863	1030/1031

Sall O2 Osym EcoRI SphI KpnI BamHI NotI

figure s2

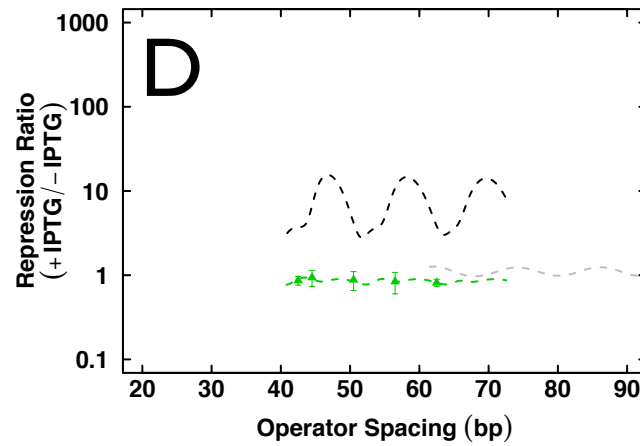
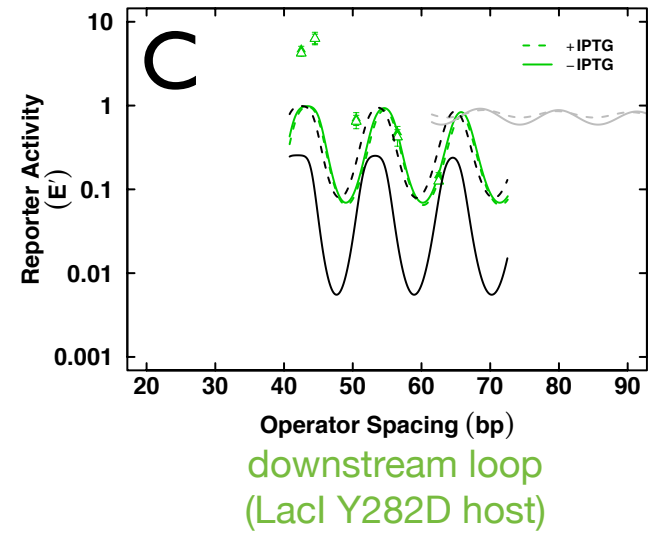
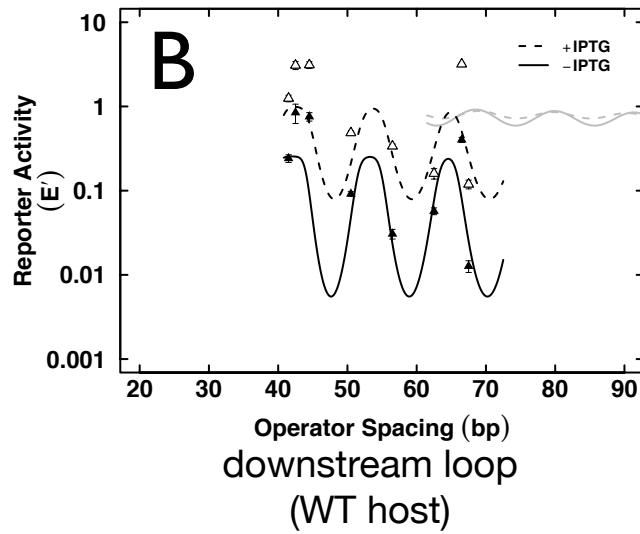
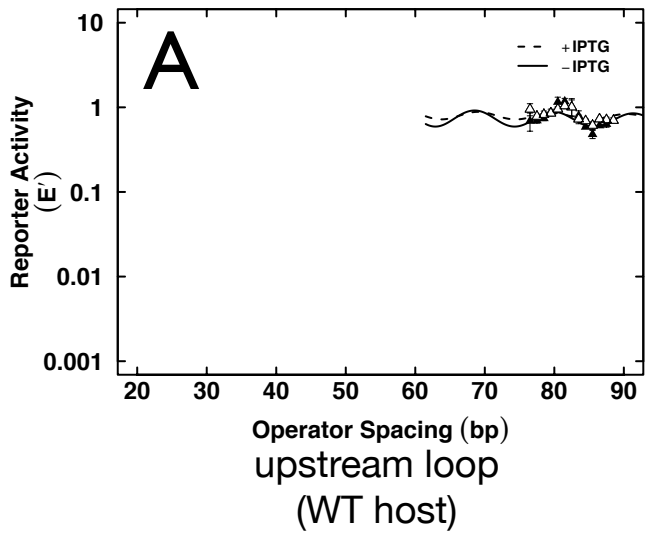
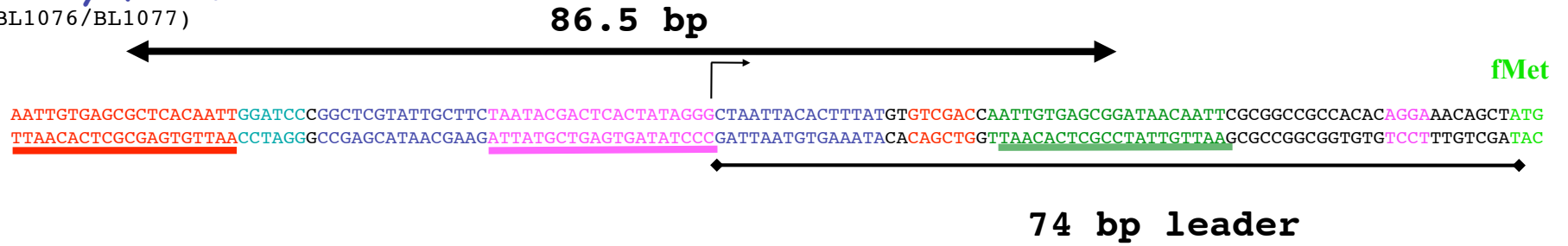


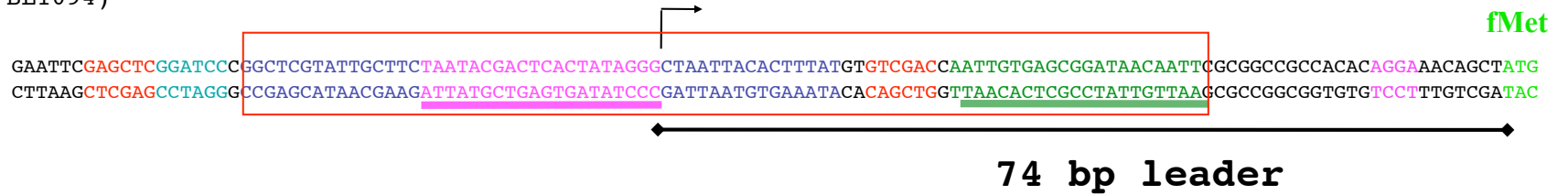
figure s3

A**Upstream Osym/O1 construct**

pJ1906 (BL1076/BL1077)

**B****O1 only construct**

pJ1938 (BL1093/BL1094)

**C****O1/Downstream Osym**

pJ1940 (BL1095/BL1096)



figure s4

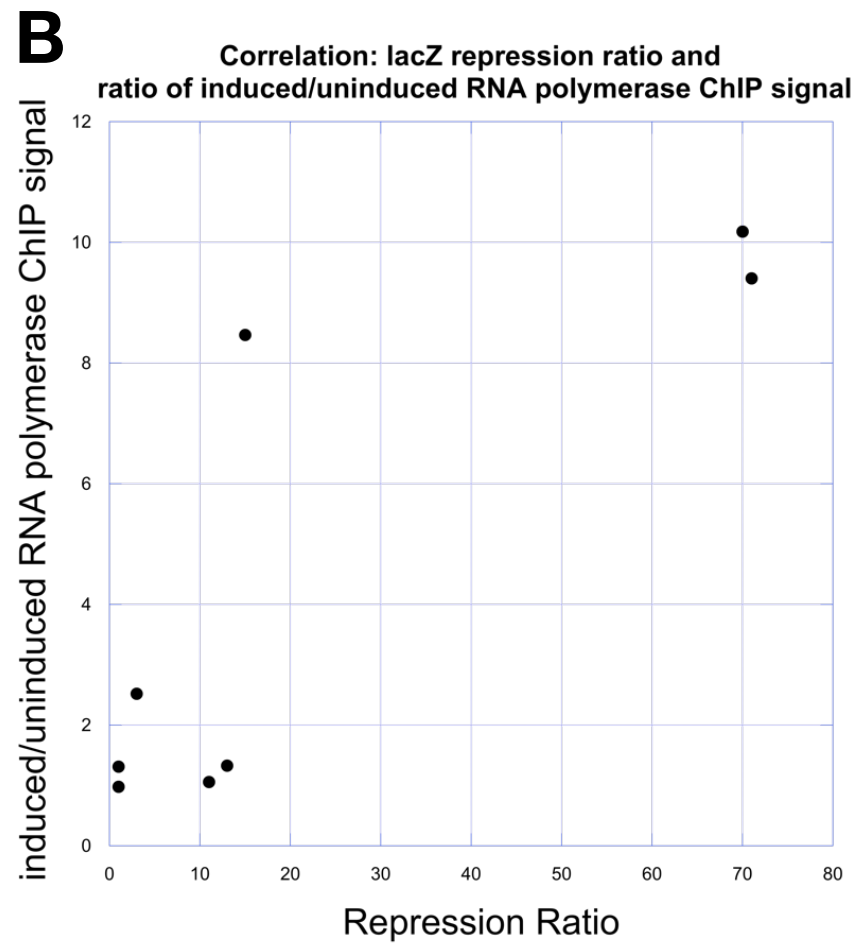
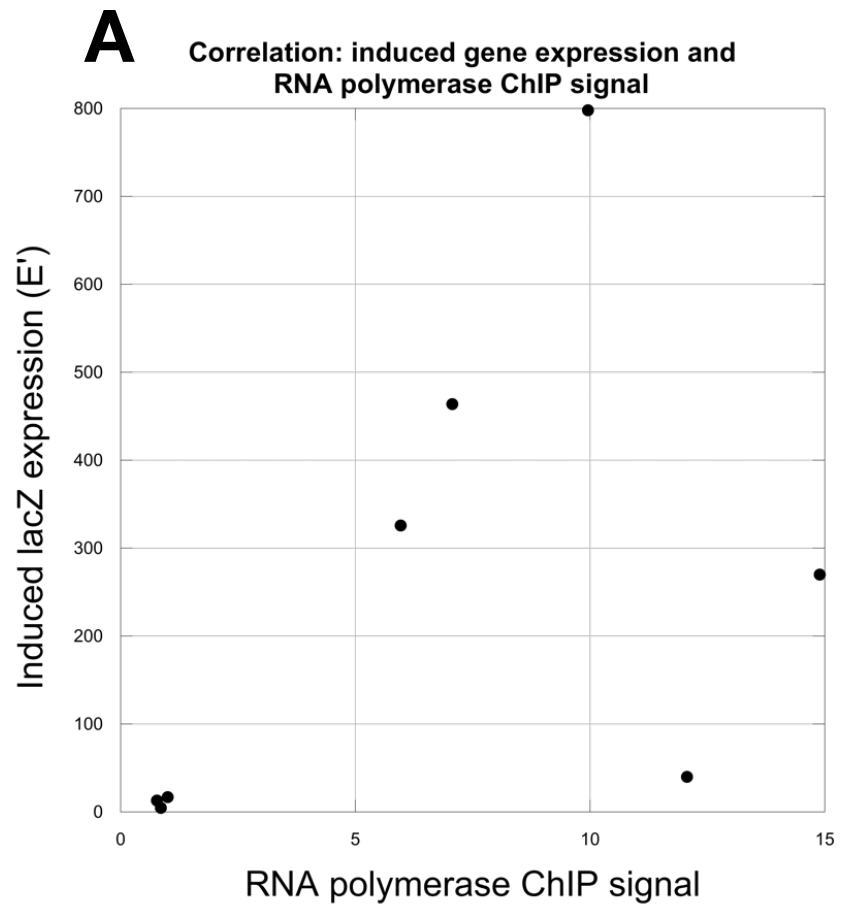


figure s5

Supplemental figure legends S1-S5:

Figure S1. *In vivo* repression behavior of different individual proximal *lac* operators in the absence of DNA looping. A. Test construct format showing position of proximal operator (green) relative to *lac* uv5 promoter. *Lac* operator sequences are shown below, with differences relative to the symmetric O_{sym} operator shown in color and lower case. The transcription start site is 3 nt upstream of the proximal operator. B. Reporter gene (*lacZ*) readout as Miller units in the absence (red) or presence (green) of IPTG inducer. C. Repression ratio based on data in B.

Figure S2. Design of promoter-reporter constructs with repression loops downstream of the promoter. A. Layout of control elements and reporter gene, showing promoter elements (-35, -10), promoter transcription start point (broken arrow), variably spaced weak and strong *lac* operators (O_2 and O_{sym} , respectively), Shine-Dalgarno sequence (sd) and *lacZ* reporter gene. B. Sequences of constructs studied in these experiments. Transcription start point (+1), restriction endonuclease cleavage sites (bottom), and center-center operator spacings (right) are indicated.

Figure S3. Repression data for promoter-reporter constructs involving single auxiliary O_{sym} operators without DNA looping. A. Induced (open triangles, dashed fit curve) and repressed (filled triangles, solid fit curve) reporter activity (E') dataset with fits to thermodynamic model for *upstream* constructs containing only an O_{sym} operator. B. Induced (open triangles, dashed fit curve) and repressed (filled triangles, solid fit curve)

reporter activity (E') dataset with fits to thermodynamic model for *downstream* constructs containing only an O_{sym} operator. C. With model fits as in (B; grey and black) but including reporter activity (E') data and model fit (green) for downstream constructs in *E. coli* lacking functional lac repressor (LacI Y282D). D. Repression ratio data fits for constructs containing only a single auxiliary O_{sym} operator upstream (dashed grey), downstream (WT lac repressor in the presence of IPTG; dashed black), and downstream (in the absence of functional lac repressor; LacI Y282D; dashed green).

Figure S4. Details of promoter-reporter constructs to analyze DNA looping effects on a T7 RNA polymerase promoter that does not overlap with lac O_1 or O_{sym} operators. A. T7 promoter (underlined, magenta) configured within a DNA loop with in-phase 86.5-bp (center-center) DNA loop between O_{sym} (underlined, red) and O_1 (underlined, green) *lac* operators. B. T7 promoter (underlined, magenta) configured upstream of lac O_1 operator (underlined, green) in the absence of DNA looping. C. T7 promoter (underlined, magenta) configured upstream of a DNA loop with in-phase 58.5-bp (center-center) DNA loop between O_1 (underlined, green) and O_{sym} (underlined, red) *lac* operators.

Figure S5. Correlations between *lacZ* gene expression and RNA polymerase chromatin immunoprecipitation (ChIP) signal. A. Correlation between IPTG-induced β -galactosidase activity and RNA polymerase ChIP signal. B. Correlation between β -galactosidase repression ratio (RR) and the ratio of induced to uninduced RNA polymerase ChIP signals.