

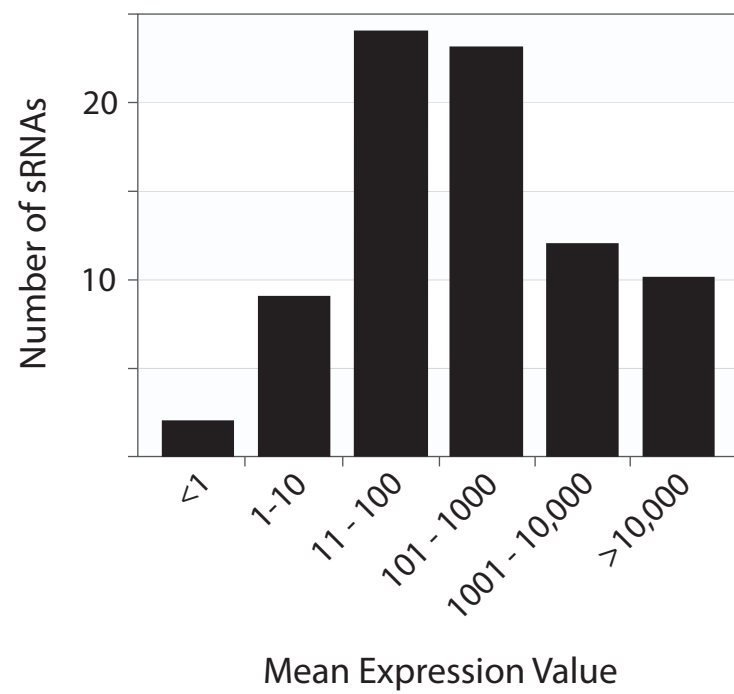
Figure S1. Distribution of Mean Expression Values (MEVs) for known sRNAs in *E. coli*.

(See Table S5 for information about individual genes.)

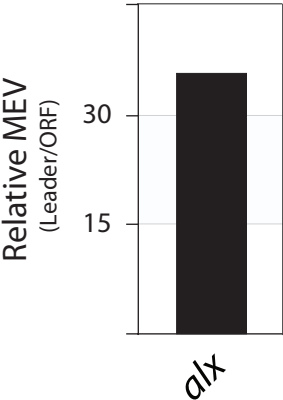
Figure S2. Transcription-termination at a pH-responsive riboregulator. **A.** Relative MEV of leader region and downstream ORF showing that the expression of the leader sequence is markedly higher than that of the *alx* gene. **B.** Expression profile of leader sequence and downstream coding region (*alx*). Y-axis denotes coverage at each nucleotide position, and X-axis shows the numerical position of transcription start site (red arrow) and translation start site (blue arrow) on the *E. coli* K12 genome (NC_000913.2).

Figure S3. Detection of novel sRNAs in intergenic regions. **A to J.** Expression profiles of intergenic regions. Y-axes denote coverage at each nucleotide, limited to a maximum of 1000 (dashed lines). Positions of transcription start sites (TSS) and terminators (stem-loop structures) found within each intergenic region are depicted. Nucleotide positions follow the numbering of the *E. coli* genome (NC_000913.2). Wide arrows on X-axes contain named ORFs (grey), sRNAs (pink), and an unnamed small ORF (green). Lengths of flanking genes not drawn to scale. Adjacent to each plot are the results of 3'-RACE experiments used to confirm the size and location of the corresponding sRNA. Start positions of primers used for 3'-RACE products are provided along with the fragment observed in each assay.

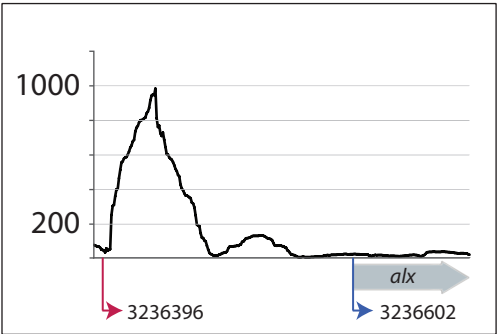
Raghavan_Fig S1



A



B



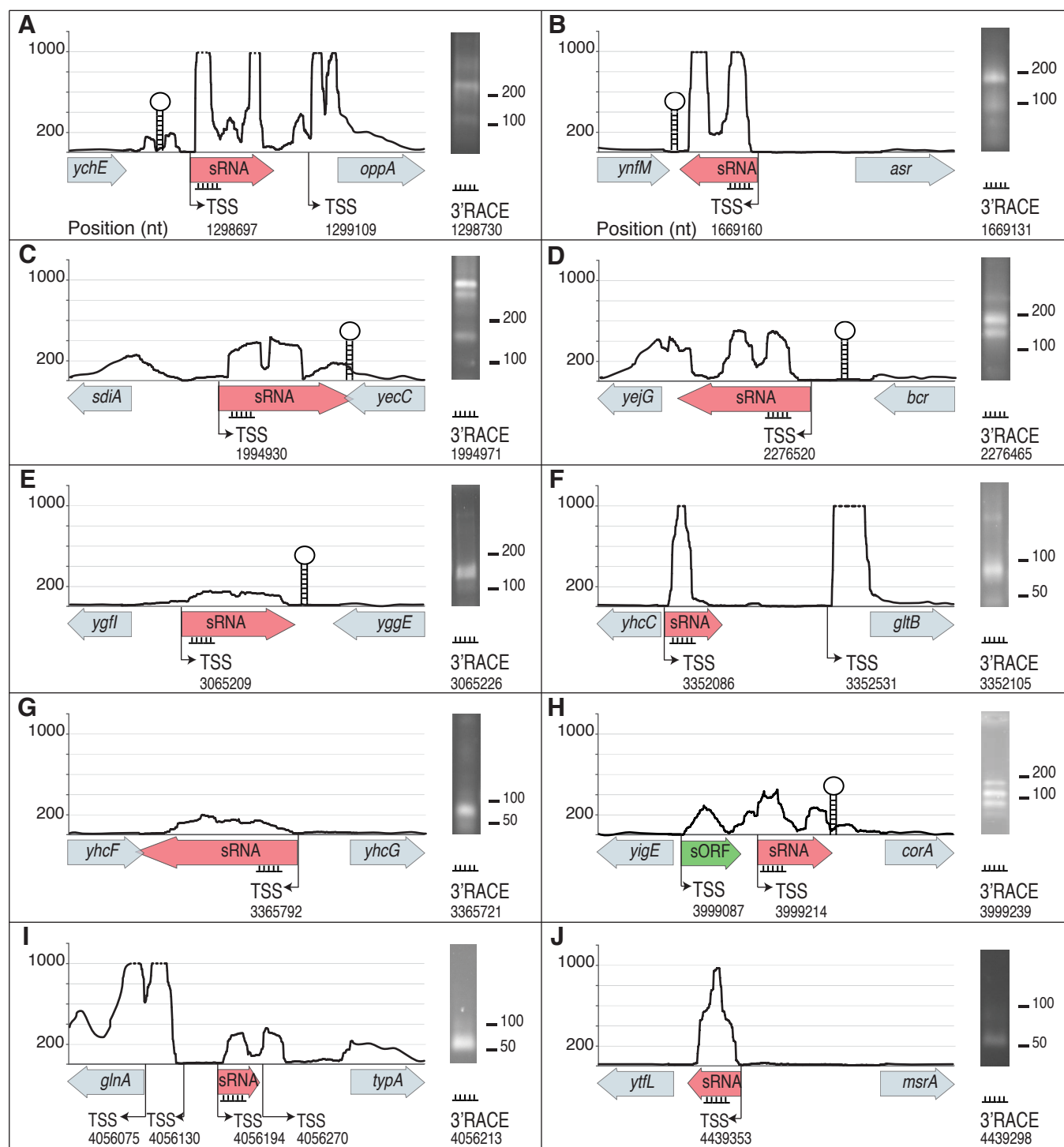


Table S1. Sequencing reads mapped to *E. coli* genome

Mg ²⁺ concentration	wild type		<i>phoP</i> ⁻	
	10mM	10uM	10mM	10uM
Total number of reads	31617192	30852852	30515441	31393591
Reads mapped to genome	31284216	30411452	30153696	30976384
Percentage mapped to 23S rRNA	2	2	3	5
Percentage mapped to 16S rRNA	1	1	1	2
Percentage mapped to 5S rRNA	69	66	58	68
Percentage mapped to tRNAs	6	7	3	7
Percentage mapped to strRNAs	4	6	5	4
Percentage mapped to ORFs	12	11	18	8
Percentage mapped to IGRs	6	8	11	6

^a RNAs other than rRNAs and tRNAs annotated in NC_000913.2

Table S2. Abundance of PhoP-regulated transcripts in *E. coli*

	wild-type		<i>phoP</i> ⁻	
	Mg ²⁺ concentration			
	10 mM	10μM	10 mM	10μM
<i>mgrR</i>	3070	21335	24	47
<i>mgtA</i> leader ^a	1.6	44	0.5	0.8

^a Transcript abundance calculated for nucleotides beyond the intrinsic termination point (corresponding to positions 180 to 262 in Fig. S3 in Cromie et al. 2006)

Table S3. sRNAs levels that correlate with PhoP and/or with Mg²⁺ levels ^a

PhoP repressed and Mg²⁺ independent		
sRNA	Relative abundance (PhoP-/PhoP+) ^b	Relative abundance (High Mg ²⁺ /Low Mg ²⁺) ^c
<i>isrC</i>	5.89	1.08
<i>sokX</i>	2.39	0.76
PhoP activated and Mg²⁺ independent		
sRNA	Relative abundance (PhoP+/PhoP-)	Relative abundance (Low Mg ²⁺ /High Mg ²⁺)
<i>gcvB</i>	2.49	1.17
PhoP activated and Mg²⁺ dependent		
sRNA	Relative abundance (PhoP+/PhoP-)	Relative abundance (Low Mg ²⁺ /High Mg ²⁺)
<i>glmY</i>	2.22	2.26
PhoP independent and Mg²⁺ dependent		
sRNA	Relative abundance (PhoP-/PhoP+)	Relative abundance (High Mg ²⁺ /Low Mg ²⁺)
<i>sraA</i>	1.28	2.45
<i>eyeA</i>	1.28	4.25

<i>ryjB</i>	1.07	2.72
sRNA	Relative abundance (PhoP-/PhoP+)	Relative abundance (Low Mg ²⁺ /High Mg ²⁺)
<i>rprA</i>	1.66	4.88

^a Only a relative abundance $\geq 2X$ is considered upregulated.

^b Wild-type *E. coli* MG1655 strain and *phoP*-deleted strain EG12976, respectively.

^c MgCl₂ concentrations of 10 mM and 10 μ M, respectively.

Table S4. Putative regulatory leader sequences detected by sequence and secondary structure conservation

Transcription start site ^a	ORF start site ^a	Gene	Length (nt)	Putative motifs (RibEx) ^b	RNAz score ^c
89367	89634	<i>mraZ</i>	268	RLE0006	–
398672	398817	<i>yaiZ</i>	146	RLE0228	–
2267840	2268001	<i>spr</i> ^d	162	RLE0356	–
2998172	2997913	<i>ygeR</i>	259	RLE0241	–
3572984	3572901	<i>asd</i>	83	RLE0311	–
3735289	3735200	<i>bax</i>	89	RLE0284	0.96
4056272	4056430	<i>typA</i> ^e	159	RLE0212	0.88
4173308	4173411	<i>thrU</i>	104	RLE0146	0.94
4437540	4437610	<i>ytfK</i>	71	RLE0172	–
4440213	4440405	<i>ytfM</i>	193	RLE0185	–

^a Numbering according to *E. coli* MG1655 (NC_000913.2)

^b Abreu-Goodger and Merino 2005.

^c Values of P, as calculated by RNAz (Gruber et al. 2007)

^d Previously predicted by Chen et al. 2002

^e Previously predicted by Rivas et al. 2001

Table S5. Expression of known sRNAs

Left_end ^a	Right_end ^a	Length	sRNA	MEV ^b	PRM ^c
14081	14169	89	<i>tpke11</i>	18.3	100
16952	17006	55	<i>sokC</i>	95.6	100
75516	75608	93	<i>sroA</i>	244.0	100
77367	77593	227	<i>sgrS</i>	177.4	96
122859	123017	159	<i>tp2</i>	2981.2	100
189712	189847	136	<i>tff</i>	1744.4	100
271804	271878	75	<i>eyeA</i>	4.3	86
457952	458008	57	<i>sraA</i>	9.7	100
475672	475785	114	<i>ffs</i>	5689.9	100
506428	506511	84	<i>sroB</i>	74.3	100
606957	607015	59	<i>sokE</i>	41.6	100
685904	686066	163	<i>sroC</i>	0	0
852175	852263	89	<i>rybA</i>	5044.6	100
887199	887277	79	<i>rybB</i>	54	100
1145812	1145980	169	<i>psrD</i>	507	100
1195937	1196009	73	<i>c0293</i>	23.3	99
1229852	1229930	79	<i>c0299</i>	1.5	70
1268546	1268612	67	<i>rdlA</i>	38.2	100
1269081	1269146	66	<i>rdlB</i>	76.7	100
1269616	1269683	68	<i>rdlC</i>	200	100
1286289	1286459	171	<i>rttR</i>	204.7	100
1403676	1403833	158	<i>isrA</i>	144.4	100
1407153	1407274	122	<i>fmrS</i>	2659.4	100
1407387	1407461	75	<i>c0343</i>	20.2	100
1435145	1435253	109	<i>micC</i>	1.7	54
1489467	1489530	64	<i>rydC</i>	36.1	100
1490143	1490198	56	<i>sokB</i>	70.9	100
1620841	1620938	98	<i>mgrR</i>	12202.8	100
1647406	1647458	53	<i>dicF</i>	3.1	70
1762737	1762804	68	<i>rydB</i>	197.5	100
1768396	1768501	106	<i>rprA</i>	46.2	100
1886041	1886126	86	<i>sroD</i>	48.2	99
1921090	1921338	249	<i>ryeA</i>	1163.7	100
1921188	1921308	121	<i>ryeB</i>	641	100
1985863	1986022	160	<i>isrB</i>	4909.9	100
2023251	2023337	87	<i>dsrA</i>	19.7	91
2031673	2031763	91	<i>rseX</i>	0.4	40
2069339	2069542	204	<i>isrC</i>	7.1	90
2151333	2151475	143	<i>sibA</i>	194.5	100

2151668	2151803	136	<i>sibB</i>	533.4	100
2165138	2165224	87	<i>cyaR</i>	16.6	100
2311106	2311198	93	<i>micF</i>	15	100
2494651	2495085	435	<i>tpke70</i>	86.6	100
2638617	2638708	92	<i>sroE</i>	131.7	100
2651537	2651745	209	<i>is128</i>	59	98
2651877	2652180	304	<i>ryfA</i>	194	100
2689179	2689362	184	<i>glmY</i>	40053.3	100
2698081	2698399	319	<i>ryfB</i>	424.9	99
2698542	2698618	77	<i>ohsC</i>	1.3	58
2732175	2732317	143	<i>ryfD</i>	15455.8	100
2753615	2753977	363	<i>ssrA</i>	12037.9	100
2812824	2812901	78	<i>micA</i>	219.8	100
2885376	2885431	56	<i>sokX</i>	5.4	91
2922178	2922537	360	<i>csrB</i>	15107.8	100
2940718	2940923	206	<i>gcvB</i>	30569.1	100
2974124	2974211	88	<i>omrA</i>	27.9	100
2974326	2974407	82	<i>omrB</i>	29.7	100
3054005	3054187	183	<i>ssrS</i>	56151	100
3054871	3055010	140	<i>sibC</i>	828.7	100
3119380	3119601	222	<i>c0719</i>	35.1	100
3182592	3182740	149	<i>sroG</i>	1949	100
3192745	3192887	143	<i>sibD</i>	12568.9	100
3193121	3193262	142	<i>sibE</i>	4988.4	100
3236396	3236583	188	<i>psrN^d</i>	289.6	100
3268238	3268614	377	<i>rnpB</i>	1799.9	100
3309247	3309420	174	<i>psrO</i>	241.8	100
3348599	3348719	121	<i>arcZ</i>	127.1	100
3578950	3579039	90	<i>ryhB</i>	76537	100
3662887	3662991	105	<i>gadY</i>	1282.9	100
3698159	3698224	66	<i>rdlD</i>	87.9	100
3720099	3720128	30	<i>sokA</i>	20.8	100
3851141	3851280	140	<i>istR2</i>	5.1	93
3984455	3984626	172	<i>glmZ</i>	524.3	99
4047922	4048030	109	<i>spf</i>	21533.5	100
4049059	4049303	245	<i>csrC</i>	9202.3	94
4156308	4156417	110	<i>oxyS</i>	140.6	85
4188350	4188510	161	<i>sroH</i>	6981.2	100
4275950	4276089	140	<i>ryjA</i>	593.6	98
4526000	4526089	90	<i>ryjB</i>	475.3	100
4577858	4577934	77	<i>symR</i>	141.5	100

^aNumbering according to *E. coli* MG1655 (NC_000913.2) of left and right boundaries of sRNAs

^b MEV, Mean Expression Value

^c PRM, Percentage Region Mapped

^d *psrN* has been shown to be a pH-responding regulatory leader sequence upstream of *alx* gene (Nechooshtan et al. 2009) (See figure S2 for its expression profile)

Table S6. Intergenic regions (IGRs) in *E. coli* with substantial transcripts

Left end ^a	Right end ^a	Orientation ^b	MEV ^c	Undefined TSSs ^d
11787	12162	Divergent	34	
57110	57363	Divergent	191	
63265	63428	Codirectional_R	69	
122857	123016	Codirectional_F	3425	
131261	131614	Divergent	191	
146695	146967	Divergent	1	+
259325	259611	Divergent	9	+
344216	344627	Codirectional_F	69	+
395512	395862	Divergent	36	+
398558	398816	Divergent	22	+
440568	440772	Divergent	184	+
611718	612037	Divergent	495	
623734	624107	Divergent	103	
637797	638167	Divergent	65	
702835	703166	Divergent	12	
712026	712209	Divergent	120	+
728045	728356	Divergent	5	+
849321	849672	Divergent	132	
882612	882895	Divergent	87	
889043	889311	Divergent	78	+
915271	915695	Divergent	15	+
931274	931817	Divergent	94	
1050399	1050683	Divergent	3535	+
1062999	1063258	Divergent	11	
1194175	1194345	Divergent	440	
1276842	1277179	Divergent	28	
1297345	1297820	Divergent	186	+
1324666	1324875	Codirectional_F	111	+
1416254	1416694	Divergent	4	
1432282	1432981	Codirectional_R	211	+
1445308	1445542	Divergent	27	+
1521090	1521330	Divergent	123	
1524005	1524270	Divergent	8	+
1630310	1631062	Divergent	149	+
1638610	1639362	Codirectional_R	1975	+
1639579	1639878	Divergent	2530	+
1642329	1642674	Codirectional_R	47	+

1644762	1645197	Convergent	71	+
1647459	1647632	Codirectional_F	38	+
1668977	1669399	Codirectional_F	271	+
1671526	1671936	Divergent	289	+
1735570	1735867	Divergent	310	
1739147	1739436	Codirectional_F	609	
1800595	1801117	Divergent	108	+
1903284	1903711	Codirectional_F	6	+
1984153	1984948	Divergent	7	
2051353	2051666	Divergent	23	+
2085139	2085352	Codirectional_R	389	+
2226781	2227003	Divergent	1	
2244792	2245084	Codirectional_R	2779	
2288203	2288521	Divergent	9	
2310772	2311105	Divergent	89	
2342192	2342886	Divergent	13	
2463030	2463322	Divergent	76	
2468485	2468836	Codirectional_F	1873	+
2481362	2481776	Divergent	33	
2583548	2583752	Divergent	48	+
2759196	2759372	Convergent	114	+
2763176	2763534	Codirectional_R	36	
2798498	2798744	Codirectional_F	1358	+
2997914	2998366	Divergent	29	+
3065196	3065361	Codirectional_R	123	+
3107178	3107574	Divergent	53	+
3119302	3119655	Codirectional_R	32	
3198988	3199228	Divergent	104	
3208566	3208802	Divergent	693	
3219270	3219487	Divergent	7	
3352073	3352653	Divergent	250	+
3365665	3365848	Codirectional_F	101	+
3372512	3372890	Divergent	1	
3376674	3376891	Codirectional_R	2690	+
3399110	3399413	Codirectional_R	282	
3401355	3401505	Divergent	96	+
3403054	3403457	Codirectional_F	234	
3426785	3427257	Divergent	3524	
3451293	3451529	Codirectional_R	3864	
3474463	3474628	Codirectional_R	161	
3483841	3484141	Divergent	715	
3530462	3530839	Divergent	40	
3572902	3573093	Divergent	74	+

3595584	3596006	Divergent	57	
3598807	3599050	Codirectional_R	1010	
3628626	3628990	Codirectional_R	440	+
3637744	3638133	Divergent	143	
3645857	3646550	Divergent	311	
3655591	3656388	Codirectional_F	196	
3667212	3667614	Divergent	5	
3735201	3735519	Divergent	9	+
3833953	3834244	Divergent	8	
3851281	3851575	Divergent	55	
3865446	3865750	Divergent	71	
3881753	3882358	Divergent	1239	
3939351	3939830	Divergent	3333	
3964114	3964439	Codirectional_F	1577	
3984031	3984454	Divergent	3	
3988790	3989175	Divergent	703	
3999080	3999448	Divergent	210	+
4083846	4084038	Divergent	358	+
4116114	4116537	Divergent	104	+
4124834	4125035	Divergent	2127	
4152871	4153023	Divergent	138	
4205556	4206169	Divergent	2521	
4254490	4254659	Divergent	67	
4258027	4258343	Divergent	3	
4273065	4273493	Divergent	66	+
4285395	4285786	Divergent	20	
4328262	4328524	Codirectional_F	479	
4366351	4366686	Divergent	29	
4380342	4380665	Divergent	90	
4422815	4423140	Divergent	188	
4437286	4437609	Divergent	25	+
4440200	4440404	Divergent	54	+
4498815	4499282	Divergent	13	+
4499613	4500125	Convergent	160	+
4537525	4538979	Divergent	17	
4554344	4555015	Divergent	20	
4558704	4558952	Divergent	3	
4566543	4567020	Divergent	17	+
4600882	4601499	Divergent	8	

^aNumbering according to *E. coli* MG1655 (NC_000913.2) of left and right boundaries of IGRs

^bF, forward strand, and R, reverse strand; according to EcoGene (Rudd 2000)

^cMEV, Mean Expression Value

^dIGRs with transcripts originating from TSSs not described in EcoCyc (Keseler et al. 2009)

Table S7. Detection of previously predicted sRNAs by RNA-Seq

Name	Left end ^a	Right end ^a	Length	Strand ^b	MEV ^c	PRM ^d	Source ^e
IS001	20666	20798	132	R	56	98	2
tp2	122859	123017	159	F	2981	100	1
IS009	302835	303034	199	F	15	91	2
p14	402834	402927	94	R	18	100	1
IS011	450965	451296	331	F	8	97	2
IS014	480028	480388	360	R	76	93	2
IS018	573636	573747	111	F	60	100	2
IS019	576356	576507	151	F	21	55	2
IS022	584964	585220	256	F	13	100	2
IS023	623829	624038	209	R	123	100	2
tp28	709961	710158	198	R	293	100	1
IS029	752270	752396	126	F	5	75	2,3
c-8	770261	770500	240	F	142	77	4
NC021	854993	855137	145	F	9	97	3
k2	914218	914570	353	F	22	95	1
IS038	915522	915655	133	F	33	100	2
tk5	921522	921589	68	F	34	78	1
IS042	1014724	1014910	186	R	568	97	2
IS055	1223195	1223288	93	F	19	100	2
tp21	1255837	1255942	106	F	7	87	1,3
t29	1415412	1415512	101	F	17	100	1
NC044	1565327	1565515	189	F	7	96	3
NC048	1636282	1636395	114	R	10	94	3
IS077	1644975	1645147	172	R	105	100	2
IS078	1669041	1669213	172	R	414	97	2,3
tp29	1903368	1903613	246	F	6	54	1
te10	1976232	1976427	196	F	34	100	1
IS094	1994969	1995088	119	F	285	100	2
NC059	2040100	2040182	83	F	2	84	3
IS109	2190861	2190980	119	F	18	100	2
IS116	2411266	2411418	152	F	110	100	2
IS118	2468479	2468609	130	R	1871	100	2,3
k27	2720644	2720746	103	F	75	100	1
IS135	2759200	2759301	101	F	32	80	2
NC087	2796832	2796960	129	R	2	83	3
IS141	2876309	2876478	169	R	44	100	2
IS143	2925871	2926018	147	F	11	96	2

IS149	3048695	3048955	260	R	50	95	2
tpke85	3081833	3081949	117	F	96	100	1
NC093	3098850	3098919	70	F	3	91	3
tpk16	3107239	3107413	175	F	66	100	1
te21	3326110	3326261	152	R	383	100	1
IS166	3358753	3358963	210	R	5	80	2
IS173	3517316	3517406	90	R	211	100	2
IS174	3537814	3538009	195	R	8	94	2
tp46	3679988	3680184	197	F	49	100	1
IS190	3904607	3904724	117	R	3	62	2
tpke120	3999150	3999449	300	F	184	100	1,2
p1	4049881	4050062	182	F	25	98	1
tpk3	4056084	4056430	347	F	229	100	1
p2	4404011	4404207	197	R	384	100	1
IS219	4501648	4501912	264	F	5	84	2
tpke10	4531814	4532310	497	F	73	100	1

Predicted candidates that overlap sRNAs identified using RNA-Seq (Table 2) are shown in bold

^aNumbering according to *E. coli* MG1655 (NC_000913.2) of left and right boundaries of sRNAs

^bF, forward strand; R, reverse strand

^cMEV, Mean Expression Value

^dPRM, Percentage of Region Mapped

^eData from previous reports: 1, Rivas et al. 2001; 2, Chen et al. 2002; 3, Yachie et al. 2006; 4, Tran et al. 2009.

Table S8. Previously predicted sRNAs associated with the 5' leader sequence of genes

Left end ^a	Right end ^a	Length	MEV ^b	PRM ^c	Gene	Source ^d
230998	231122	125	20	80	<i>yafD</i>	1
450836	451032	197	164	100	<i>cyoA</i>	1
460468	460675	208	741	100	<i>hupB</i>	1
661867	661975	109	144	100	<i>ybeD</i>	1
829880	830077	198	54	93	<i>ybiH</i>	1
932314	932446	133	169	100	<i>ftsK</i>	1
938568	938651	84	376	100	<i>serS</i>	1
950305	950479	175	26	95	<i>pflA</i>	1
960253	960424	172	28	88	<i>cmk</i>	1
986207	986403	197	580	100	<i>ompF</i>	1
1005016	1005137	122	119	100	<i>ymdB</i>	1
1146775	1146874	100	464	100	<i>plsX</i>	1
1165110	1165308	199	46	78	<i>ndh</i>	1
1739222	1739379	157	732	100	<i>cfa</i>	2
1804162	1804289	128	21	35	<i>ydiY</i>	3
1860602	1860795	194	181	100	<i>gapA</i>	1
1886017	1886085	69	96	100	<i>rnd</i>	1
2163583	2163691	109	42	100	<i>yegQ</i>	1
2267591	2267950	359	355	100	<i>spr</i>	2
2481604	2481798	195	99	88	<i>evgA</i>	3
2496319	2496477	159	44	100	<i>yfdZ</i>	1
2496321	2496528	207	35	100	<i>yfdZ</i>	2
2576422	2576639	217	28	100	<i>maeB</i>	2
2702137	2702356	220	61	100	<i>rnc</i>	1
2729426	2729619	194	1925	90	<i>rrsG</i>	1
2744209	2744457	249	316	100	<i>rpsP</i>	1
2821793	2821871	79	349	100	<i>recA</i>	1
2870844	2871029	186	204	100	<i>ftsB</i>	1
2937334	2937389	56	38	100	<i>fucR</i>	1
2995624	2995711	88	20	100	<i>insC</i>	1
3048691	3048918	228	59	96	<i>gcvT</i>	1
3071715	3071957	243	251	100	<i>epd</i>	1
3079665	3079899	234	52	60	<i>yggG</i>	5
3086121	3086340	220	6	96	<i>galP</i>	4
3154542	3154761	220	4	98	<i>dkgA</i>	1
3184115	3184209	95	19	100	<i>insC</i>	1
3208607	3208803	197	3178	94	<i>rpsU</i>	1
3309708	3309848	141	6964	100	<i>rpsO</i>	1

3376675	3376886	212	1603	100	<i>rplM</i>	1
3399181	3399240	60	108	100	<i>mreB</i>	4
3440495	3440640	146	794	100	<i>rpsM</i>	1
3446173	3446336	164	1501	100	<i>rplN</i>	1
3451294	3451526	233	2352	100	<i>rpsJ</i>	1
3472576	3472700	125	653	100	<i>rpsL</i>	1
3474476	3474627	152	117	87	<i>yheO</i>	1
3483881	3484140	260	645	100	<i>crp/yhfA</i>	4
3517372	3517479	108	49	100	<i>aroK</i>	1
3597740	3597855	116	41	92	<i>livJ</i>	1
3598854	3599051	198	801	100	<i>rpoH</i>	1
3604407	3604474	68	26	100	<i>zntA</i>	1
3655593	3655904	312	313	97	<i>gadE</i>	3
3770015	3770304	290	5	83	<i>mtlA</i>	1
3809713	3809910	198	2041	100	<i>rpmB</i>	1
3850809	3850913	105	87	100	<i>ilvB</i>	1
4176314	4176470	157	577	100	<i>rplK</i>	1
4183298	4183373	76	27	100	<i>rpoC</i>	1
4213201	4213520	320	208	65	<i>aceB</i>	4
4225651	4225767	117	30	100	<i>yjbB</i>	1
4328267	4328511	245	398	100	<i>proP</i>	1
4363381	4363492	112	27	91	<i>cutA</i>	1
4465463	4465647	185	282	98	<i>mgtA</i>	1
4532011	4532320	310	56	100	<i>yjhX</i>	1
4566576	4566830	255	22	100	<i>mdtM</i>	1
4566653	4566793	140	24	100	<i>mdtM</i>	2
4610026	4610434	409	82	97	<i>ytjA</i>	1

^aNumbering according to *E. coli* MG1655 (NC_000913.2) of left and right boundaries of IGRs

^bMEV, Mean Expression Value

^cPRM, Percentage of Region Mapped

^dData from previous reports: 1, Rivas et al. 2001; 2, Chen et al. 2002; 3, Yachie et al. 2006; 4, Tran et al. 2009; 5, Argaman et al. 2001.