

Supplemental Material for:

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The *Bacillus subtilis* σ^M Regulon and its Contribution to Cell Envelope Stress Responses.

Table S1. Plasmids used in this work

Table S2. Strains used in this work

Table S3. Oligonucleotides used in this work

Table S4. Pixel intensities for σ^M -ROMA experiment

Table S1. Plasmids used in this work.

pJPM122	Vector for integration into SP β	Slack et al. 1991
pWE01	pET16 carrying <i>NcoI/BamHI sigM</i> coding region	This work
pWE02	<i>PypbG-cat-lacZ</i> in pJPM122	This work
pWE03	<i>PyfnI-cat-lacZ</i> in pJPM122	This work
pWE04	<i>Pmaf-cat-lacZ</i> in pJPM122	This work
pWE05	<i>PyebC-cat-lacZ</i> in pJPM122	This work
pWE06	<i>ProdA-cat-lacZ</i> in pJPM122	This work
pWE07	<i>PypuD-cat-lacZ</i> in pJPM122	This work
pWE08	<i>PyngC-cat-lacZ</i> in pJPM122	This work
pWE09	<i>PywtF-cat-lacZ</i> in pJPM122	This work
pWE10	<i>PycgR-cat-lacZ</i> in pJPM122	This work
pWE11	<i>PydaH-cat-lacZ</i> in pJPM122	This work
pWE12	<i>PmurG-cat-lacZ</i> in pJPM122	This work
pWE13	<i>Psms-cat-lacZ</i> in pJPM122	This work
pWE14	<i>PypuA-cat-lacZ</i> in pJPM122	This work
pWE15	<i>PspX-cat-lacZ</i> in pJPM122	This work
pWE16	<i>PrecU-cat-lacZ</i> in pJPM122	This work
pWE17	<i>PydbO-cat-lacZ</i> in pJPM122	This work
pWE18	<i>PsecDF-cat-lacZ</i> in pJPM122	This work
pWE19	<i>PmetA-cat-lacZ</i> in pJPM122	This work
pWE20	<i>PyacA-cat-lacZ</i> in pJPM122	This work
pWE21	<i>PytnA-cat-lacZ</i> in pJPM122	This work
pWE22	<i>PytpA-cat-lacZ</i> in pJPM122	This work

Table S2. Strains used in this study

Strain	Genotype	Reference
<i>E. coli</i>		
DH5 α	<i>supE44</i> Δ <i>lacU169</i> (ϕ 80 <i>lacZ</i> Δ <i>M15</i>) <i>hsdR17</i> <i>recA1</i> <i>endA1</i> <i>gyrA96</i> <i>thi-1</i> <i>relA1</i> 32	Lab stock
BL21/DE3	BL21 with λ DE3 (T7 RNAP under <i>lac</i> control)	Lab stock
HE4600	<i>E. coli</i> BL21/DE3(pWE01)	This work
<i>B. subtilis</i>		
CU1065	W168 <i>attSPβ</i> <i>trpC2</i>	Lab stock
ZB307A	W168 SP β c2 β Δ 2::Tn917::pBSK10 Δ 6 (MLS ^R)	Lab stock
HB0031	CU1065 <i>sigM</i> :: <i>kan</i>	Lab stock
HB4715	CU1065 <i>sigM</i> :: <i>tet</i> <i>sigW</i> :: <i>m</i> ls <i>sigX</i> :: <i>spc</i>	This work
HB4728	CU1065 <i>spx</i> :: <i>spc</i>	This work
HB0069	CU1065 SP β (<i>Pm-cat-lacZ</i>)	Lab stock
HB0070	HB0031 SP β (<i>Pm-cat-lacZ</i>)	Lab stock
HB4682	ZB307A with SP β (<i>PypbG-cat-lacZ</i>)	This work
HB4684	ZB307A with SP β (<i>PyfnI-cat-lacZ</i>)	This work
HB4687	ZB307A with SP β (<i>Pmaf-cat-lacZ</i>)	This work
HB4689	ZB307A with SP β (<i>PyebC-cat-lacZ</i>)	This work
HB4681	ZB307A with SP β (<i>ProdA-cat-lacZ</i>)	This work
HB4665	ZB307A with SP β (<i>PypuD-cat-lacZ</i>)	This work
HB4686	ZB307A with SP β (<i>PyngC-cat-lacZ</i>)	This work
HB0078	ZB307A with SP β (<i>PywaC-cat-lacZ</i>)	Lab stock
HB4708	ZB307A with SP β (<i>PywtF-cat-lacZ</i>)	This work
HB7026	ZB307A with SP β (<i>PyrhH-cat-lacZ</i>)	Lab stock
HB4664	ZB307A with SP β (<i>PycgR-cat-lacZ</i>)	This work
HB4710	ZB307A with SP β (<i>PydaH-cat-lacZ</i>)	This work
HB4736	ZB307A with SP β (<i>PmurG-cat-lacZ</i>)	This work
HB4683	ZB307A with SP β (<i>PytmA-cat-lacZ</i>)	This work
HB4738	ZB307A with SP β (<i>Psms-cat-lacZ</i>)	This work
HB4734	ZB307A with SP β (<i>PypuA-cat-lacZ</i>)	This work
HB4762	ZB307A with SP β (<i>PspX-cat-lacZ</i>)	This work
HB4730	ZB307A with SP β (<i>PrecU-cat-lacZ</i>)	This work
HB4733	ZB307A with SP β (<i>PydbO-cat-lacZ</i>)	This work
HB4737	ZB307A with SP β (<i>PsecDF-cat-lacZ</i>)	This work
HB4729	ZB307A with SP β (<i>PmetA-cat-lacZ</i>)	This work
HB4732	ZB307A with SP β (<i>PyacA-cat-lacZ</i>)	This work
HB4735	ZB307A with SP β (<i>PytpA-cat-lacZ</i>)	This work
HB0075	ZB307A with SP β (<i>PyjbC-cat-lacZ</i>)	Lab stock
HB4690	CU1065 SP β (<i>PypbG-cat-lacZ</i>)	This work
HB4696	CU1065 SP β (<i>PyfnI-cat-lacZ</i>)	This work
HB4698	CU1065 SP β (<i>Pmaf-cat-lacZ</i>)	This work
HB4700	CU1065 SP β (<i>PyebC-cat-lacZ</i>)	This work
HB4702	CU1065 SP β (<i>ProdA-cat-lacZ</i>)	This work
HB4670	CU1065 SP β (<i>PypuD-cat-lacZ</i>)	This work
HB4706	CU1065 SP β (<i>PyngC-cat-lacZ</i>)	This work
HB4660	CU1065 SP β (<i>PywaC-cat-lacZ</i>)	This work
HB4711	CU1065 SP β (<i>PywtF-cat-lacZ</i>)	This work
HB7034	CU1065 SP β (<i>PyrhH-cat-lacZ</i>)	Lab stock
HB4668	CU1065 SP β (<i>PycgR-cat-lacZ</i>)	This work
HB4717	CU1065 SP β (<i>PydaH-cat-lacZ</i>)	This work

HB4739	CU1065 SPβ (<i>PmurG-cat-lacZ</i>)	This work
HB4704	CU1065 SPβ (<i>PymA-cat-lacZ</i>)	This work
HB4745	CU1065 SPβ (<i>Psms-cat-lacZ</i>)	This work
HB4749	CU1065 SPβ (<i>PypuA-cat-lacZ</i>)	This work
HB4763	CU1065 SPβ (<i>PspX-cat-lacZ</i>)	This work
HB4754	CU1065 SPβ (<i>PrecU-cat-lacZ</i>)	This work
HB4756	CU1065 SPβ (<i>PydbO-cat-lacZ</i>)	This work
HB4741	CU1065 SPβ (<i>PsecDF-cat-lacZ</i>)	This work
HB4752	CU1065 SPβ (<i>PmetA-cat-lacZ</i>)	This work
HB4743	CU1065 SPβ (<i>PyacA-cat-lacZ</i>)	This work
HB4760	CU1065 SPβ (<i>PytpA-cat-lacZ</i>)	This work
HB4758	CU1065 SPβ (<i>PyjbC-cat-lacZ</i>)	This work
HB4691	HB0031 SPβ (<i>PypbG-cat-lacZ</i>)	This work
HB4697	HB0031 SPβ (<i>PyfnI-cat-lacZ</i>)	This work
HB4699	HB0031 SPβ (<i>Pmaf-cat-lacZ</i>)	This work
HB4701	HB0031 SPβ (<i>PyebC-cat-lacZ</i>)	This work
HB4703	HB0031 SPβ (<i>ProdA-cat-lacZ</i>)	This work
HB4671	HB0031 SPβ (<i>PypuD-cat-lacZ</i>)	This work
HB4707	HB0031 SPβ (<i>PyngC-cat-lacZ</i>)	This work
HB4661	HB0031 SPβ (<i>PywaC-cat-lacZ</i>)	This work
HB4712	HB0031 SPβ (<i>PywtF-cat-lacZ</i>)	This work
HB4718	HB0031 SPβ (<i>PyrhH-cat-lacZ</i>)	This work
HB4669	HB0031 SPβ (<i>PycgR-cat-lacZ</i>)	This work
HB4716	HB0031 SPβ (<i>PydaH-cat-lacZ</i>)	This work
HB4740	HB0031 SPβ (<i>PmurG-cat-lacZ</i>)	This work
HB4705	HB0031 SPβ (<i>PymA-cat-lacZ</i>)	This work
HB4746	HB0031 SPβ (<i>Psms-cat-lacZ</i>)	This work
HB4750	HB0031 SPβ (<i>PypuA-cat-lacZ</i>)	This work
HB4764	HB0031 SPβ (<i>PspX-cat-lacZ</i>)	This work
HB4755	HB0031 SPβ (<i>PrecU-cat-lacZ</i>)	This work
HB4757	HB0031 SPβ (<i>PydbO-cat-lacZ</i>)	This work
HB4742	HB0031 SPβ (<i>PsecDF-cat-lacZ</i>)	This work
HB4753	HB0031 SPβ (<i>PmetA-cat-lacZ</i>)	This work
HB4744	HB0031 SPβ (<i>PyacA-cat-lacZ</i>)	This work
HB4761	HB0031 SPβ (<i>PytpA-cat-lacZ</i>)	This work
HB4759	HB0031 SPβ (<i>PyjbC-cat-lacZ</i>)	This work
HB4708	HB4715 SPβ (<i>PypuD-cat-lacZ</i>)	This work
HB4709	HB4715 SPβ (<i>PycgR-cat-lacZ</i>)	This work
HB4710	HB4715 SPβ (<i>ProdA-cat-lacZ</i>)	This work
HB4711	HB4715 SPβ (<i>Pmaf-cat-lacZ</i>)	This work
HB4712	HB4715 SPβ (<i>PyebC-cat-lacZ</i>)	This work
HB4713	HB4715 SPβ (<i>PywtF-cat-lacZ</i>)	This work
HB4765	HB4715 SPβ (<i>PsecDF-cat-lacZ</i>)	This work
HB4767	HB4715 SPβ (<i>PypuA-cat-lacZ</i>)	This work
HB4768	HB4715 SPβ (<i>PyjbC-cat-lacZ</i>)	This work
HB4769	HB4715 SPβ (<i>PmetA-cat-lacZ</i>)	This work

Table S3. Oligonucleotides used in this study

Number	Name	Sequence (5'-3')
3037	<i>sigM</i> fwd (pET16b)	ataccatggtgacgatcgatgaaatttacc
3038	<i>sigM</i> rev (pET16b)	ataggatcctttcctggcgcgtcatttcc
3153	<i>sigM</i> -for -in	ggtatattccattgtgccc
3154	<i>sigM</i> -rev -in	gctgtgatcataggagtg
3251	<i>ycgR</i> fwd (pJPM122)	cccaagcttcacatgcacatcatgcaaaaag
3252	<i>ycgR</i> rev (pJPM122)	cgggatccgaatggcttctattaaaaatg
3253	<i>ypuD</i> fwd (pJPM122)	cccaagcttctaccogtttaacgaaatgc
3254	<i>ypuD</i> rev (pJPM122)	cgggatccgtacatataaccgcctgcaag
3388	<i>yebC</i> fwd (pJPM122)	cccaagcttgctggttgtcgtatttatcc
3389	<i>yebC</i> rev (pJPM122)	cgggatccgcatctattctttcactctg
3390	<i>ywtF</i> fwd (pJPM122)	cccaagcttgcaatgacattgtctgtggg
3391	<i>ywtF</i> rev (pJPM122)	cgggatccgcccgaatgactaaaaaagc
3392	<i>rodA</i> fwd (pJPM122)	cccaagcttcaaattctattggatggcg
3393	<i>rodA</i> rev (pJPM122)	cgggatccgcagcatatattgatactac
3448	<i>ypbG</i> fwd (pJPM122)	cccaagcttcgcagcccgataaatggaag
3349	<i>ypbG</i> rev (pJPM122)	cgggatccgctggtgcatacatttttgc
3850	<i>murG</i> fwd (pJPM122)	cccaagcttcacgaacaaaacagcctgcc
3851	<i>murG</i> rev (pJPM122)	cgggatcccttccgcccgaataaagac
3442	<i>yfnI</i> fwd (pJPM122)	cccaagcttggtattcttatactcgggat
3443	<i>yfnI</i> rev (pJPM122)	cgggatcccagtataacagccagcaciaa
3444	<i>ytnA</i> fwd (pJPM122)	cccaagcttctggcacagcatcaatattg
3445	<i>ytnA</i> rev (pJPM122)	cgggatccgaggaacaaccctactccta
3446	<i>yngC</i> fwd (pJPM122)	cccaagcttccgaaatgagatctcagctt
3447	<i>yngC</i> rev (pJPM122)	cgggatccgataacaccgcaataattg
3474	<i>maf</i> fwd (pJPM122)	cccaagcttcagctgcctactccattatt
3475	<i>maf</i> rev (pJPM122)	cgggatccgcagatcgtgatgaccaag
3468	<i>ydaH</i> fwd (pJPM122)	cccaagcttgctgacttggcggatgata
3558	<i>ydaH</i> rev (pJPM122)	cgggatccgtaatgacatgcacgttatcc
3863	<i>sms</i> fwd	cccaagcttgctcatcaaacagtgctcctg
3864	<i>sms</i> rev	cgggatccctggagcagatgtaatatcg
3865	<i>ypuA</i> fwd	cccaagcttcaaaggagattatgtatagg
3866	<i>ypuA</i> rev	cgggatccggaacctcagcagcaaaac
3852	<i>spx</i> fwd	cccaagcttgagaaatggtacggagaatc
3898	<i>spx</i> rev	cgggatcccagtatcctaagaaaaaat
3867	<i>recU</i> fwd	cccaagcttcatgcagcagatgaaacagc
3878	<i>recU</i> rev	cgggatcccggattgagcggcagacaaa
3869	<i>ydbO</i> fwd	cccaagcttctgaagcgtgagagcagac
3870	<i>ydbO</i> rev	cgggatcccattgcccgcgataagatttgg
3871	<i>secDF</i> fwd	cccaagcttcagataagaagtggaaaaag
3872	<i>secDF</i> rev	cgggatccccgatcaatagaacgaaaag
3873	<i>metA</i> fwd	cccaagcttgaactcataagaaacctaac
3874	<i>metA</i> rev	cgggatccggttactttcaagcacctgt
3875	<i>yacA</i> fwd	cccaagcttctataaaaagcatcaccgcag
3876	<i>yacA</i> rev	cgggatcccgctacaatgattgtcgcc
3823	<i>ytpA</i> fwd	cccaagcttccgtctaaaatgattgagcc
3824	<i>ytpA</i> rev	cgggatccccgtgaatgattacaatcac
3364	<i>ypbG</i> -gsp1	gatgttttctcgtattctcg
3365	<i>ypbG</i> -gsp2	cctcagctaaatcaccgccc

3368	ywaC-gsp1	cggcaatgtcgtgaatatgc
3369	ywaC-gsp2	cacagccctttctcattaac
3378	rodA-gsp1	ctgtaatggctacagcccc
3379	rodA-gsp2	caatccgtgttcccatactg
3486	yfnI-gsp1	ccagcaaagccagtcctaaa
3487	yfnI-gsp2	ggcgcttgagaatgggttaa
3488	ypuD-gsp1	gatactctgagccgtcaatt
3489	ypuD-gsp2	gccgtcaattttcgtcactt
3490	maf-gsp1	ctcttccttggatgccgtat
3491	maf-gsp2	gccgtatgcacctgctttat
3492	yngC-gsp1	cttcccgtatttgtccagaa
3493	yngC-gsp2	gcgaccgatccagtaaataa
3494	ydaH-gsp1	ccgactgtcgatccgaaaat
3495	ydaH-gsp2	ctacaatcgccagtgcgttt
3406	ytnA-gsp1	ggccatttcccgaagcgctc
3407	ytnA-gsp2	ccgcttgcggcatacgtac
3848	murG-gsp1	ggctctggccacaatgacat
3849	murG-gsp2	ctcaggcatttgatgaagaa
3410	ycgR-gsp1	gcaacgcccgcgatgcagagg
3411	ycgR-gsp2	gcctcgtaatcgggatgatg
3374	yebC-gsp1	cgcaagcttgtctttaacag
3375	yebC-gsp2	gttctctggcgcggtttctgc
3376	ywF-gsp1	gcgtgcaaggctgacatgag
3377	ywF-gsp2	ctttacaaaagcataggcgc
3846	yacA-gsp1	cctctgaaccgatggtcaac
3847	yacA-gsp2	cggccgcataacgtatgcaa
3836	recU-gsp1	gtcattccgcgattactgta
3837	recU-gsp2	ctgactgttttgggatgaaa
3842	ytpA-gsp1	ggcaacaggtctgtctgctt
3843	ytpA-gsp2	ccacatgatcttcacctcat
3840	secDF-gsp1	ctgaacgcgaatccggttat
3841	secDF-gsp2	gttcgctgacaccgagaaca
3838	ydbO-gsp1	gatatcccacgcagttttgc
3839	ydbO-gsp2	ggatcaaccacggcagtt
3844	metA-gsp1	cgtgtgtgtgcttgggatta
3845	metA-gsp2	gcaatttccaagcaatctg
3819	spx-up-fwd	cgagtactttccaattgaag
3820	spx-up-rev (spec)	cgttacgttatttagcgagccagtcgcatgaagtacagcttgggtg
3821	spx-do-for (spec)	caataaaccttgcctcgctacggagaagcacagcgtttggca
3822	spx-do-rev	cttcatggagcatgactatc

Table S4. Pixel intensities for transcripts generated by E σ^M vs. “core” alone

All genes with a >30 pixel increase are shown; sorted by signal intensity; **bold**= σ^M regulon; *italic*=readthrough from upstream σ^M -dependent promoter.

Name	gene position	E-sigM	core	Difference in pixel intensities
spx	1227169	2513	137	2376
<i>mecA</i>	1228540	2290	113	2177
ywaC	3948746	1665	6	1659
yebC	695704	1574	13	1561
divIC	69166	1711	182	1529
abh	1517270	1062	23	1039
sigM	1029057	1024	2	1022
rodA	3911128	1019	4	1015
<i>ywtE</i>	3694420	1007	5	1002
maf	2861833	828	6	821
ywtF	3693296	767	7	760
<i>rapA</i>	1315338	9233	8493	740
yqjL	2476198	712	6	705
<i>abrB</i>	44846	626	61	565
<i>yycD</i>	4158213	543	7	536
yhdL	1027991	529	1	528
yrhI	2776340	499	2	497
yfnI	795780	454	8	445
ywbO	3924592	433	6	427
yhdK	1027713	432	21	411
ydaH	474284	398	2	396
<i>yebG</i>	697601	391	4	386
ysxA	2861101	373	0	373
ywbN	3925478	389	23	366
mreB	2859997	324	11	313
<i>nfrA</i>	3910273	306	17	289
divIB	1593100	348	72	275
minD	2857038	293	19	274
<i>yycC</i>	4158785	293	19	274
dltA	3951069	282	21	261
<i>ywcH</i>	3909098	231	2	229
<i>ykqB</i>	1519936	243	17	226
<i>gspA</i>	3943354	252	35	217
<i>ugtP</i>	2305756	224	8	216
<i>murAA</i>	3777004	328	113	214
<i>yhdI</i>	1025735	208	1	207
<i>yebE</i>	697050	203	1	202
yrhJ	2773160	211	10	201
<i>spoIID</i>	3775777	285	84	201
bcrC	3757601	228	28	200

<i>yebD</i>	696666	192	2	190
<i>kinC</i>	1517738	196	8	189
yngC	1946923	174	9	165
metA	2304619	165	1	165
pbpX	1765226	170	12	158
mreC	2859094	159	8	151
<i>purC</i>	701106	149	7	142
yceC	311713	187	51	137
<i>ykqA</i>	1519040	135	4	131
<i>ywcD</i>	3920687	140	14	126
ytpA	3121743	128	8	120
<i>ymdA</i>	1766678	434	317	117
murB	1592058	153	40	113
ytnA	3123331	114	5	109
<i>nth</i>	2343997	115	6	109
minC	2857846	114	8	107
<i>ftsA</i>	1595870	145	39	106
<i>topB</i>	476111	103	2	101
recU	3796140	101	2	99
<i>yycB</i>	4158998	111	14	97
<i>ydaJ</i>	478497	96	1	95
<i>yocL</i>	2096948	95	4	91
<i>lrpC</i>	475612	90	-1	91
<i>yocK</i>	2096382	115	25	90
murG	1590940	111	22	89
<i>dnaD</i>	2344675	89	0	89
<i>ydbR</i>	510663	120	32	88
<i>asnS</i>	2345466	90	3	87
<i>ywcC</i>	3921088	85	-1	85
<i>ywbC</i>	3935929	126	41	85
<i>purK</i>	698602	87	4	83
<i>purB</i>	699738	81	-2	83
<i>ypmB</i>	2348106	85	2	83
ylxX	1594606	112	31	82
<i>ftsZ</i>	1597228	196	114	82
yceG	314437	99	19	80
ddl	507805	88	9	79
<i>yycA</i>	4160311	81	3	78
yceD	312334	165	91	74
<i>ywaF</i>	3944324	72	-1	74
<i>mobA</i>	1494910	75	4	71
<i>fdhD</i>	3772421	102	31	71
<i>ywmC</i>	3774708	101	32	70
sbp	1595331	89	20	69
<i>ywsB</i>	3706201	205	138	68
<i>ywcI</i>	3905808	104	38	66
<i>phoA</i>	1016366	67	2	65
<i>galK</i>	3919434	69	5	64
<i>ytxJ</i>	3045831	344	281	63
<i>sacT</i>	3904938	146	86	60
<i>lysC</i>	2908623	62	2	60
<i>ask</i>	2910025	291	232	59

dltB	3952577	65	7	58
yrS	2788190	119	61	58
yqzJ	2756759	66	10	56
yceE	312950	171	115	56
murF	508941	63	7	56
adeC	1520756	59	5	54
yfnH	797977	55	2	54
ywmD	3773710	82	29	53
yocM	2097572	60	7	53
yrhP	2767312	59	6	53
yacA	74927	79	27	52
spoIIE	70536	71	19	52
yfhB	922125	55	3	51
ybfN	249584	54	4	50
yjbG	1230738	59	9	50
ywnH	3759748	54	5	50
yxjJ	3995621	370	324	47
purS	701824	47	1	46
yfnG	798811	55	9	46
ysmB	2903144	53	8	45
yheE	1049923	99	54	45
mreD	2858579	50	6	44
spoIVFA	2856093	46	2	44
purL	702075	45	2	43
ybfM	248257	59	17	42
ymdB	1768309	55	13	42
yrvD	2825111	50	9	41
secDF	2826129	42	1	40
yrvE	2822684	44	4	40
disA	107473	118	79	40
yabS	73104	175	136	39
cotF	4166318	62	23	39
yabT	73930	46	7	39
ponA	2340686	53	15	38
yqjG	2483133	47	9	38
rplU	2854779	82	45	38
yceH	316066	49	13	37
ydaL	480428	35	-1	36
rapK	2061405	53	17	36
moaA	3771380	63	27	36
psd	248738	44	8	36
yrvC	2825510	38	3	35
spoIVFB	2855234	38	3	35
ydaM	482130	35	1	35
acoR	883264	93	59	34
ftsH	76982	76	43	33
ypbG	2403483	36	3	33
rpmA	2854141	696	663	33
ysxB	2854438	300	267	33
purF	704946	35	3	33
rbsD	3702328	52	19	33
dltE	3955286	44	11	33

dltD	3954017	40	8	32
yqhH	2549051	40	8	32
ytzC	3118646	56	24	32
ywsA	3706893	38	6	32
dltC	3953781	41	10	32
yhdE	1012867	61	30	31
purQ	702742	33	2	31
ypdP	2308972	32	1	31
ypdQ	2309661	34	3	31
ymaC	1862690	34	4	31