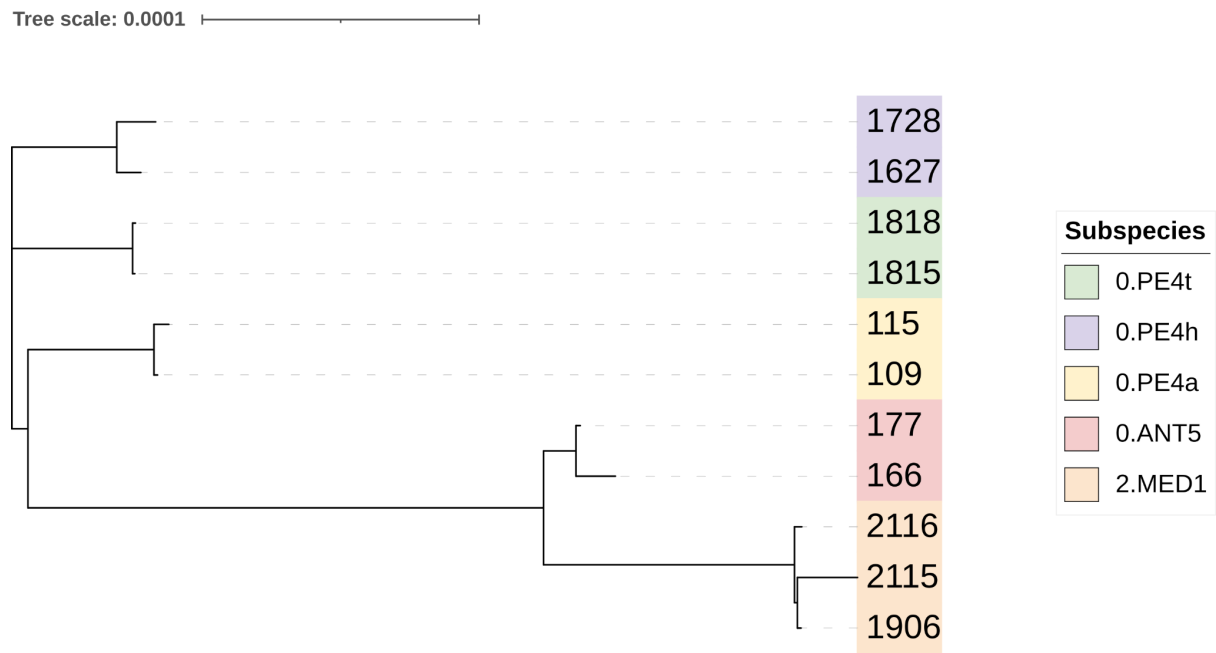




Supplementary Figure 1. Phylogenetic tree of the strains used in this study. The tree was constructed based on the co-aligned core genomes of the studied strains.

Supplementary Table 1. The list of *Y. pestis* strains used in the study.

№	Strain	Isolation source, date	Isolation place	Subspecies, biovar, phylogenetic line branch
1	177	<i>Marmota baibacina</i> , 08.09.2019	Upper-Naryn high-mountain plague focus, Kyrgyz Republic	<i>Y. pestis</i> subsp. <i>pestis</i> (bv. antiqua) 0.ANT5
2	166	<i>Marmota baibacina</i> , 25.11.2020	Sarydzhas high mountain plague focus, Kyrgyz Republic	<i>Y. pestis</i> subsp. <i>pestis</i> (bv. antiqua) 0.ANT5
3	1906	<i>Meriones meridianus</i> 12.05.2014	Caspian sandy plague focus, Republic of Kalmykia, Russia	<i>Y. pestis</i> subsp. <i>pestis</i> (bv. medievalis) 2.MED1
4	2115	<i>Meriones meridianus</i> 31.10.2014	Caspian sandy plague focus, Republic of Kalmykia, Russia	<i>Y. pestis</i> subsp. <i>pestis</i> (bv. medievalis) 2.MED1
5	2116	<i>Xenopsylla conformis</i> 03.11.2014	Caspian sandy plague focus, Republic of Kalmykia, Russia	<i>Y. pestis</i> subsp. <i>pestis</i> (bv. medievalis) 2.MED1
6	1815	<i>Ceratophyllus caspius</i> 06.09.1980	Talas high-mountain plague focus, Kyrgyz Republic	<i>Y. pestis</i> subsp. <i>central asiatica</i> (bv. talassica) 0.PE4t
7	1818	<i>Alticola argentatus</i> 28.08.1980	Talas high-mountain plague focus, Kyrgyz Republic	<i>Y. pestis</i> subsp. <i>central asiatica</i> (bv. talassica) 0.PE4t
8	1728	<i>Alticola argentatus</i> 09.07.1972	Hissar high-mountain plague focus, Republic of Tajikistan	<i>Y. pestis</i> subsp. <i>central asiatica</i> (bv. hissarica) 0.PE4h
9	1627	<i>Neodon juldaschi</i> 08.06.1971	Hissar high-mountain plague focus, Republic of Tajikistan	<i>Y. pestis</i> subsp. <i>central asiatica</i> (bv. hissarica) 0.PE4h
10	109	<i>Ctenophyllus hirticrus</i> 29.04.2019	Gorno-Altai high mountain plague focus, Altai Republic, Russia	<i>Y. pestis</i> subsp. <i>central asiatica</i> (bv. altaica) 0.PE4a
11	115	<i>Paramonopsyllus scalonae</i> 29.04.2019	Gorno-Altai high mountain plague focus, Altai Republic, Russia	<i>Y. pestis</i> subsp. <i>central asiatica</i> (bv. altaica) 0.PE4a

¹Cadastre of epidemic and epizootic manifestations of plague in the territory of the Russian Federation and neighboring countries (1876–2015). Edited by Kutyrev VV, Popova AY. Saratov: “Amirit”. 2016

²Kutyrev VV, Eroshenko GA, Motin VL, Nosov NY, Krasnov JM, Kukleva LM, Nikiforov KA, Al'khova ZV, Oglodin EG, Guseva NP. Phylogeny and Classification of *Yersinia pestis* Through the Lens of Strains From the Plague Foci of Commonwealth of Independent States. Front Microbiol. 2018 May 25;9:1106. doi: 10.3389/fmicb.2018.01106. PMID: 29887859; PMCID: PMC5980970.

Supplementary Table 2. Sequencing depth of the samples used in the study. The strains used for visualization in the main text are marked by green.

Phylogenetic line	strain	22C, median sequencing depth	37C, median sequencing depth
0.PE4a	109	246X	85X
	115	168X	282X
0.ANT5	166	123X	141X
	177	195X	107X
0.PE4h	1627	248X	83X
	1728	167X	43X
0.PE4t	1815	226X	256X
	1818	216X	96X
2.MED1	1906	111X	423X
	2115	200X	257X
	2116	154X	163X

Supplementary Table 3. The percentage of GATC and CCWGG occurrences with methylation level > 50%.

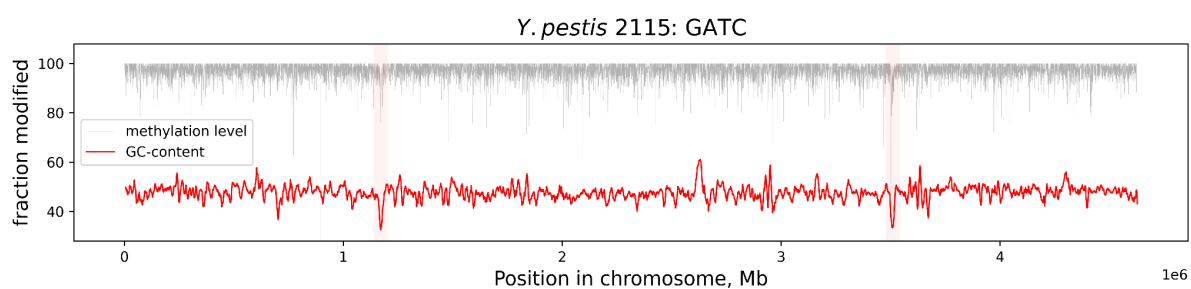
Phylogenetic line	Strain	GATC methylation, %		CCWGG methylation, %	
		22C	37C	22C	37C
0.PE4a	109	99.973	99.125	23.263	1.027
	115	99.965	99.947	20.705	1.431
0.ANT5	166	99.973	99.962	25.943	12.014
	177	99.976	99.937	22.824	6.305
0.PE4h	1627	99.960	99.956	22.583	0.735
	1728	99.968	99.895	23.842	1.674
0.PE4t	1815	99.962	99.288	28.116	2.687
	1818	99.96	99.907	29.703	2.343
2.MED1	1906	99.972	99.944	21.782	10.06
	2115	99.973	99.976	23.463	16.164
	2116	99.981	99.968	20.547	13.653

Supplementary Table 4A. Under-methylation of GATC in two AT-rich chromosomal regions.

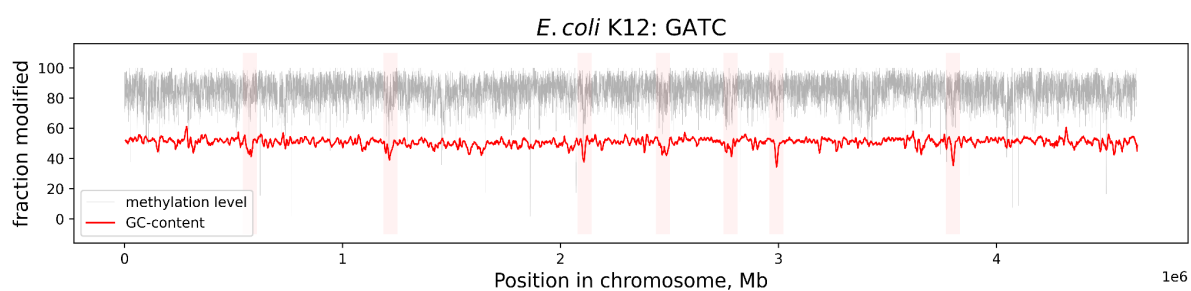
	O-antigen biosynthesis operon				Type II secretion system			
	22C		37C		22C		37C	
Strain	log10 p-val	effect size	log10 p-val	effect size	log10 p-val	effect size	log10 p-val	effect size
109	-16	-1.1	-9	-0.77	-16	-1.28	-8	-1.05
115	-14	-0.97	-19	-1.01	-11	-1.15	-15	-1.18
166	-8	-0.62	-16	-0.88	-7	-0.71	-4	-0.69
177	-23	-1.32	-8	-0.65	-11	-1.18	-23	-1.37
1627	-15	-0.89	-20	-1.06	-12	-0.93	-14	-1.03
1728	-12	-0.9	-7	-0.57	-8	-0.8	-10	-0.94
1815	-19	-1.08	-15	-0.95	-16	-1.11	-12	-1.34
1818	-22	-0.95	-10	-0.65	-16	-1.2	-16	-1.11
1906	-14	-0.83	-16	-0.98	-8	-0.72	-11	-1.01
2115	-21	-1.21	-17	-1.05	-14	-1.32	-14	-1.24
2116	-11	-0.93	-10	-0.74	-12	-1.12	-13	-1.08

Supplementary Table 4B. Under-methylation of CCWGG in two AT-rich chromosomal regions.

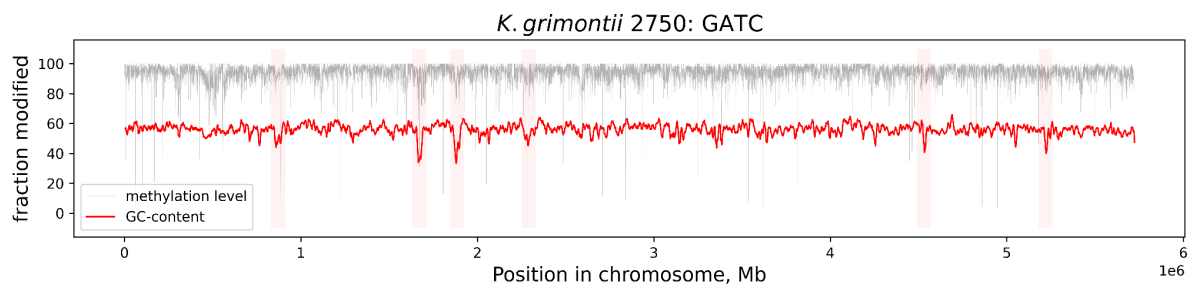
	O-antigen biosynthesis operon				Type II secretion system			
	22C		37C		22C		37C	
Strain	log10 p-val	effect size	log10 p-val	effect size	log10 p-val	effect size	log10 p-val	effect size
109	-3.6	-0.78	-0.7	-0.33	-0.5	-0.22	-0.1	-0.03
115	-3.5	-0.76	-1.2	-0.44	-0.4	-0.18	-0.6	-0.37
166	-3.9	-0.81	-3.7	-0.71	-1.4	-0.44	-0.6	-0.24
177	-3.8	-0.82	-2.6	-0.64	-0.6	-0.27	-0.7	-0.25
1627	-3.7	-0.77	-1.3	-0.37	-0.6	-0.27	-0.4	-0.2
1728	-3.1	-0.74	-1.1	-0.44	-0.7	-0.32	-0.4	-0.35
1815	-3.2	-0.73	-0.5	-0.26	-0.6	-0.25	-0.8	-0.08
1818	-3.3	-0.76	-2.3	-0.57	-0.6	-0.27	-0.2	-0.03
1906	-4.5	-0.89	-3.5	-0.71	-1.0	-0.36	-1.0	-0.32
2115	-4.0	-0.83	-3.4	-0.73	-0.6	-0.29	-0.3	-0.2
2116	-4.2	-0.83	-3.9	-0.78	-0.5	-0.21	-0.5	-0.21



Supplementary Figure 2. Under-methylation of GATC sites in AT-rich regions: *Y. pestis* 2115.



Supplementary Figure 3. Under-methylation of GATC sites in AT-rich regions: *E. coli* K12.



Supplementary Figure 4. Under-methylation of GATC sites in AT-rich regions: *K. grimontii* 2750.

Supplementary Table 5. Calculated Differential Methylation (DM) scores for the genes identified as differentially methylated in adenine between 22°C and 37°C samples. Positive values indicate over-methylation in the 22°C sample compared to the 37°C sample. Values close to zero mean that methylation does not significantly differ between samples. Actually, only the *pla* gene demonstrated significant change in adenine methylation, but only in two of the considered strains.

Phylogenetic line	strain	<i>pla</i>	<i>cmpD</i>
0.PE4a	109	24.65	0.584
	115	2.277	2.364
0.ANT5	166	0.675	-0.142
	177	0.369	-0.17
0.PE4h	1627	2.74	2.534
	1728	0.83	2.374
0.PE4t	1815	86.941	-0.008
	1818	0.115	-0.153
2.MED1	1906	-0.027	0.964
	2115	1.133	1.273
	2116	0.029	1.648

Color legend:

red cells - strong under-methylation effect (DM score > 5.0)

yellow cells - medium under-methylation effect (5.0 > DM score > 2.5)

green cells - weak under-methylation effect (2.5 > DM score > 1.0)

white cells - no significant under-methylation (DM score < 1.0)

Locus tags of the listed genes in the *Y. pestis* CO92 genome:

pla - YPO_RS00035

cmpD - YPO_RS22175

Supplementary Table 6. Calculated Differential Methylation (DM) scores for the genes identified as differentially methylated in cytosine between 22°C and 37°C samples. Positive values indicate over-methylation in the 22°C sample compared to the 37°C sample. Values close to zero mean that methylation does not significantly differ between samples.

Phylogenetic line	strain	<i>yscD</i>	<i>traI</i>	<i>parA</i>	<i>sbp</i>	<i>rpoH</i>	<i>istB</i>
0.PE4a	109	9.314	31.526	6.41	2.714	9.231	4.173
	115	47.263	9.046	19.958	19.553	26.372	14.672
0.ANT5	166	15.028	5.601	3.087	8.329	9.009	1.562
	177	43.136	19.359	14.317	4.577	3.139	3.419
0.PE4h	1627	71.532	23.82	36.148	10.157	24.412	6.607
	1728	31.551	12.821	22.709	5.59	8.332	5.856
0.PE4t	1815	65.24	84.518	23.153	10.344	21.625	7.86
	1818	171.352	70.947	25.845	22.114	17.465	33.136
2.MED1	1906	23.023	2.889	1.829	1.219	0.711	0.404
	2115	27.606	14.565	1.806	2.38	3.766	1.494
	2116	23.578	7.518	4.336	2.043	0.284	1.012

Color legend:

red cells - strong under-methylation effect (DM score > 5.0)

yellow cells - medium under-methylation effect (5.0 > DM score > 2.5)

green cells - weak under-methylation effect (2.5 > DM score > 1.0)

white cells - no significant under-methylation (DM score < 1.0)

Locus tags of the listed genes in the *Y. pestis* CO92 genome:

yscD - YPO_RS00310
traI - YPO_RS00445
parA - YPO_RS00900
sbp - YPO_RS01435
rpoH - YPO_RS20135
yajR - YPO_RS16785
dnaC - YPO_RS00055

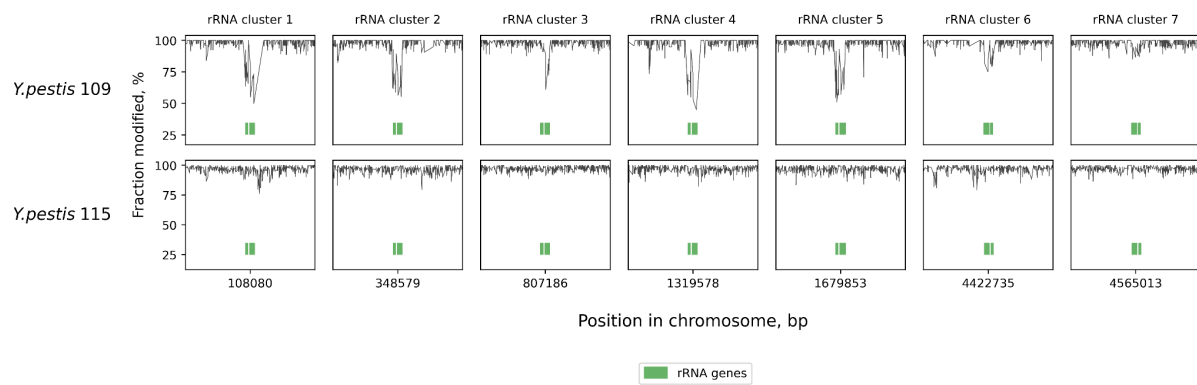
Supplementary Table 7. CCWGG under-methylation of rRNA clusters in 22C.

strain	methyated/total in genome	methyated/total in rRNA clusters
109	1524 / 6569	0 / 85
115	1359 / 6561	0 / 85
166	1709 / 6550	0 / 85
177	1491 / 6422	0 / 85
1627	1413 / 6261	0 / 85
1728	1544 / 6468	0 / 85
1815	1833 / 6519	4 / 85
1818	1858 / 6195	1 / 85
1906	1425 / 6515	0 / 85
2115	1549 / 6622	0 / 85
2116	1350 / 6591	0 / 85

Supplementary Table 8. CCWGG under-methylation of rRNA clusters in 37C.

strain	methyated/total in genome	methyated/total in rRNA clusters
109	294 / 2551*	0 / 45*
115	94 / 6575	0 / 85
166	797 / 6438	0 / 85
177	418 / 6399	0 / 85
1627	47 / 6224	0 / 85
1728	103 / 6002*	0 / 77*
1815	151 / 6145	0 / 85
1818	91 / 3395*	0 / 35*
1906	690 / 6627	0 / 85
2115	1103 / 6626	0 / 85
2116	922 / 6585	0 / 85

* only positions with sufficient coverage depth were considered (modkit n_valid > 10, --filter-threshold 0.66).



Supplementary Figure 5. Adenine under-methylation of rRNA clusters in strain 109 compared to phylogenetically close strain 115.

Supplementary Table 9. AA sequences encoded in >95% of *Y. pestis* strains but absent in *Y. pseudotuberculosis*.

protein sequence	Blast annotation
MKSRKDFTVFEYGHLLISDKDSGRINNAVSLPPQLFEWLEQRCLKDDADN THRLTLGSRGGVKTLQVKNYAGVISLPGNAFIEVLPKTGRRDENIDIAR QQLMMLRTLKSFRIHATSASRVRTSKMPLMDVFIQQFIDSQVQIVRQGL KRDYLRQQDNLPWMKGKLRVSAQISKNCVHRDRFQVEFDDYSSERPEN RILKTAIDKIRRIQSNPQLLLQVNSLQVHFDDITPVGDVRIAFDQVRLDRH MRHYELALAWARLILMGQSPHCMQGDANASILLFPM EAVFESFVTAWM RHRYNDNWHVKAQVDSHTLVRLNGKGVFKLRPDIHLRPRGENGDQAM ICDVKWKMVESGKATLEQSQADLYQMLAYGVNYQEGIGDMLLIYPSHE GFSQPLPYPEFSHQENPLRLWVVPFFIGDSLQSSDLRFPDGKILI	5-methylcytosine-specific restriction enzyme McrC
MVQDSSALASFFRSHIAKNNQYSPDWGKHRYQLVEQVQAEKPNFSADTI RQIWYERSNGISSLRQGGMSQFEFETAQKQLLALTRQMADECTQENYDR VIERLVKLKTDGILNKVYWALCHRAFAALYPSKITNVVNVSNFFSSNYC NNHFQLDLSQDEWFARNLDLKKVLHRAFGDDVDPIELNMSLWHLYTQ VIQKNDIGLIGSETALPEDEQDEEVTPQLPKNTILYGPPGTGKTYCTIEL AVRACEPAAYSLQEGKEENEKRELKKVYDRLIAEKVRFITFHQSFGYE EFIEGLRAETDDGNVRYEVKAGIFKQICEDAAFAGHAGVQQKLDEALAR LQERLSESGSITLETQGKAFQLAYKSQTTFGIFPSQSKKEDLGQGYNAY LKNISLVYQNPQAKVHNPSYVRSILNYLIKQEGLPSPNPQESVSEKRQNYV LIIDEINRGNISKIFGELITLIETSKRAGEPEALSVILPYSSSSFSVPNNLYLIG TMNTADRSLTALDTALRRRFEFEAMLDPITVLKETVVKGIDLPRLLQTLN DRIEVLVDREHTLGHAFFIPVVQVKEDEDLAERLKRIMRNKVLPLLEEY FYNDWQKIRMVLGDNQKSGNPQLQFVCEVKDQKQFADLFGNGTEDL HDIGASFHLAPEDDKVWDNPLAWQQIYAPHKGNPVSIGE	5-methylcytosine-specific restriction enzyme McrB
MLRKERSYAEIYNLDLGDVVNLFFVLRDMLRECLIEALILTPYSRDEFT DAYGEATMVERARKLVIRATMGFGSAGATKGTGFRLDTKRGSATAQ HLWARMPENLAAGQRFEGVLVENRDAVQCMLDHDVTSTLHFVDPPYV HDTRVISSRYRHEMDNDAHLTLLDTVNNLEGMVVLSGYNTDMYNDIL TGWQKQEKQSSAAGRKGSVKRLECLWLSPNVLSGSKAA	DNA adenine methyltransferase
MIKTQNAPAAIGPYSQGVVAGNLLFISGCCPFSEKDGSVVGIDITEQTIQA MKNLKAIVEATGSYMNDVVKTTCFISDMNNFQSFNITYAGYFPGSTFPAR SCVEVARLPKDVLIIEVAIVSLK	RidA family protein, partial
MGKQNFYFLGQLIGCHFNDQYAYINDGEDTIEGHIQFYKKTADEQTLSEL KKNCMILLKFILTLRRNLKRDGTGLISLPHYGKQPHMIFSKLY	Hypothetical protein

MGLNFTIPNRVSPVSRPPQNRHERVAYLCLNSDHPPLSQATPML	Hypothetical protein
MNNVLGVVIPKGIPGEQVRQLLLNDFGIEIGTSFGPLNGKIWRIGTMGYN ARKDCVMQTLVALEAVLNRLGFTTVQGAGLQATWDVYQVDSAHQVDS AHQVDSTHQADSAQRNSDHAQLN	Purine catabolism protein PucG, partial
MDNVIQMNPSPKWWSEDLMSLNGMTKHMIIQHARRKSWMEGREYIHVA PDLNPKENSPIMYNRQEIDNWVERQRPARRRIGERISA	DNA-binding protein, partial

Supplementary Table 10. Estimated plasmid copy number per cell in *Y. pestis* strains growing at different temperatures. The values are calculated as ratios of absolute coverage depth between plasmid and the chromosome.

		pMT1, estimated copy number		pCD1, estimated copy number		pPCP1, estimated copy number	
Phylogenetic line	strain	22C	37C	22C	37C	22C	37C
0.PE4a	109	1.14	1.5	2.76	3.41	12.86	17.11
	115	1.1	1.53	2.8	5.78	11.49	19.95
0.ANT5	166	1.12	1.51	2.58	5.19	14.0	30.57
	177	1.51	2.0	2.53	7.48	14.12	13.52
0.PE4h	1627	1.15	2.84	2.66	7.83	16.25	81.19
	1728	1.26	2.21	3.29	7.42	14.69	75.87
0.PE4t	1815	1.43	2.11	3.68	9.74	10.55	36.04
	1818	1.31	1.86	5.17	10.04	17.71	24.16
2.MED1	1906	1.16	1.75	2.11	6.41	5.26	18.63
	2115	1.14	1.56	2.36	6.46	6.38	19.63
	2116	0.98	1.26	2.05	6.71	9.26	14.82