

Supplementary Table 1. Details of SNP mutations in 2-20K genomes.

Clone					Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
2K	5K	10K	15K	20K							
					<i>pcnB</i>	161041	T	G	904 (302)	<u>A</u> AC→ <u>C</u> AC	Asn→His
					<i>araJ</i>	380188	A	C	717 (239)	TT <u>T</u> →TT <u>G</u>	Phe→Leu
					<i>insL-2/lon</i>	430835	C	T	−48/−108	—	—
					<i>mrdA</i>	649391	T	A	1411 (471)	<u>A</u> TC→ <u>T</u> TC	Ile→Phe
					<i>gltI</i>	668787	A	C	193 (65)	<u>T</u> AC→ <u>G</u> AC	Tyr→Asp
					<i>nagC</i>	683496	A	C	194 (65)	G <u>T</u> T→G <u>G</u> T	Val→Gly
					<i>ybjN/potF</i>	910316	T	C	+176/−175	—	—
					<i>ompF/asnS</i>	1004251	T	C	−177/+425	—	—
					<i>dhaM</i>	1248380	A	C	885 (295)	GA <u>T</u> →GA <u>G</u>	Asp→Glu
					<i>narI/ychS</i>	1286699	C	A	+23/−160	—	—
					<i>topA</i>	1329516	C	T	97 (33)	<u>C</u> AC→ <u>T</u> AC	His→Tyr
					<i>pykF</i>	1733101	G	C	137 (46)	C <u>G</u> C→C <u>C</u> C	Arg→Pro
					<i>yedW/yedX</i>	1976879	T	G	−57/−76	—	—
					<i>yegI</i>	2082685	G	A	1481 (494)	G <u>C</u> T→G <u>T</u> T	Ala→Val
					<i>insB-15</i>	2129116	A	C	268 (90)	<u>A</u> TG→ <u>C</u> TG	Met→Leu
					<i>maeB/talA</i>	2499315	G	A	−110/−179	—	—
					<i>hypF</i>	2732014	C	A	272 (91)	C <u>G</u> C→C <u>T</u> C	Arg→Leu
					<i>yghJ</i>	3045069	G	T	935 (312)	A <u>C</u> C→A <u>A</u> C	Thr→Asn
					<i>ebgR</i>	3155168	G	A	886 (296)	<u>G</u> GC→ <u>A</u> GC	Gly→Ser
					<i>tdcR/yhaB</i>	3200643	C	A	+11/−245	—	—
					<i>infB</i>	3248957	A	T	2292 (764)	GA <u>T</u> →GA <u>A</u>	Asp→Glu
					<i>arcB</i>	3288092	G	A	169 (57)	<u>C</u> GT→ <u>T</u> GT	Arg→Cys
					<i>yhdG/fis</i>	3339158	A	C	+22/−4	—	—
					<i>rpsD</i>	3369432	T	C	149 (50)	G <u>A</u> C→G <u>G</u> C	Asp→Gly
					<i>rpsM</i>	3370027	T	A	350 (117)	A <u>A</u> G→A <u>T</u> G	Lys→Met
					<i>nirC</i>	3424910	G	A	3 (1)	AT <u>G</u> →AT <u>A</u>	Met→Met*
					<i>malT</i>	3483047	C	A	1363 (455)	<u>C</u> GC→ <u>A</u> GC	Arg→Ser

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Clone					Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
2K	5K	10K	15K	20K							
					<i>glpE</i>	3490112	C	T	313 (105)	<u>G</u> CG→ <u>A</u> CG	Ala→Thr
					<i>spoT</i>	3762741	A	T	1985 (662)	A <u>A</u> →A <u>T</u> A	Lys→Ile
					<i>hslU</i>	4100655	C	T	576 (192)	AT <u>G</u> →AT <u>A</u>	Met→Ile
					<i>iclR</i>	4201911	C	T	649 (217)	<u>G</u> CG→ <u>A</u> CG	Ala→Thr
					<i>hsdM</i>	4560632	T	C	392 (131)	T <u>A</u> C→T <u>G</u> C	Tyr→Cys
					<i>nadR</i>	4615865	G	A	337 (113)	<u>G</u> CT→ <u>A</u> CT	Ala→Thr
					<i>nadR</i>	4616396	G	A	868 (290)	<u>G</u> GC→ <u>A</u> GC	Gly→Ser
3	9	16	22	29	total SNP mutations						
1	1	3	0	1	SNP mutations that are off line of descent to 40K						

Black shading indicates clones with a mutation that is on the line of descent leading to the sequenced 40K clone. Mutations off the line of descent are crosshatched. Mutations in intergenic regions have the two flanking genes listed (e.g., *ybiN/potF*). Ancestral and evolved nucleotides refer to the top strand of the genome. For mutations in genes, the nucleotide positions of the reading frame are numbered in the direction of transcription, and numbers in parentheses are the corresponding amino-acid positions. For intergenic mutations, gene positions are nucleotides relative to each of the neighboring genes, where + indicates a distance relative to the stop codon of a gene translated toward the mutation and – indicates the position of the start codon of a neighboring gene that is oriented away from the mutation. *The mutated *nirC* codon will encode Met rather than Ile if it is still utilized as an initiation codon.

Supplementary Table 2. Details of DIP mutations in 2-20K genomes.

Clone					Gene or region	Mutation type	Genome start	Genome end	Size (bp)
2K	5K	10K	15K	20K					
					<i>mokC/nhaA::IS150</i> (–)	IS-insertion§	16972	16974	3
					<i>IS1::IS150</i> (–)	IS-insertion§	241691*	241693*	3
					<i>ΔinsL-2/lon</i> –58/–98	Deletion	430845	430845	1
					<i>ybaL</i> 18 (6)	Insertion (G)	475288	475289	1
					<i>Δ(nmpC†-ECB_00513)</i>	Deletion	547700	555923	8224
					<i>inv(citC-gatZ‡)</i>	Inversion‡	634746	2128599	1493854
					ECB_00736/ECB_00737::IS186 (–)	IS-insertion§	795320	795325	6
					<i>pflB::IS150</i> (+)	IS-insertion§	969836	969838	3
					<i>xasA::IS150</i> (–)	IS-insertion§	1544289	1544291	3
					<i>pykF::IS150</i> (–)	IS-insertion§	1733647	1733649	3
					<i>ynjI::IS150</i> (–)	IS-insertion§	1821525	1821527	3
					<i>Δ(manB-cpsG)</i>	Deletion	2031703	2054995	23293
					<i>nupC/yfeA::IS186</i> (+)	IS-insertion§	2448493	2448498	6
					<i>nrdE</i> 2000 (667)	Deletion	2698099	2698099	1
					<i>kpsD::IS150</i> (–)	IS-insertion§	3015771	3015774	4
					<i>ΔgltB</i>	Deletion	3289962	3289977	16
					<i>glmU/atpC</i> –66/+286	Insertion (T)	3875632	3875633	1
					<i>kup/insJ-5</i> +6/–49	Insertion (G)	3893551	3893552	1
					<i>Δ(kup-yieO)</i>	Deletion	3894997	3901930	6934
					<i>ΔpflC</i> 342 (114)	Deletion	4126706	4126706	1
					<i>fimA::IS186</i> (+)	IS-insertion§	4524522	4524527	6
3	6	12	14	16	total DIP mutations				
1	2	3	0	1	DIP mutations that are off line of descent to 40K¶				

Black shading indicates clones with a mutation that is on the line of descent leading to the sequenced 40K clone. Mutations off the line of descent are crosshatched. Gene names and positions are as in Supplementary Table 1. †*nmpC* and *gatZ* are pseudogenes interrupted by *IS1* elements. ‡For the *IS1*-mediated inversion between *citC* and *gatZ*, start and end positions are for the region bounded by the *IS* elements. §For *IS*-element insertions, start and end positions are for the duplicated target sequence, and the resulting size corresponds to the number of target nucleotides, not the *IS* element. The orientation of the new *IS* relative to the genome's top strand is shown as + or – in the gene column. *The *IS150* insertion into an *IS1* element could have occurred in the same sequence context in any of eight different *IS1* copies. The start and end coordinates shown are for a representative example. ¶The *kup/insJ-5* insertion is present in the 10K and 20K clones but not the 15K clone. We infer that it arose independently in the lineages leading to each clone.

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>thrA</i>	1615	T	G	1280 (427)	A <u>T</u> T→A <u>G</u> T	Ile→Ser
	<i>yaaH</i>	10269	A	C	224 (75)	T <u>T</u> C→T <u>G</u> C	Phe→Cys
	<i>ECB_00021/ECB_00022</i>	21936	T	G	−161/+272	–	–
	<i>carB</i>	35850	A	C	963 (321)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>caiA</i>	44151	T	G	429 (143)	AG <u>A</u> →AG <u>C</u>	Arg→Ser
	<i>caiT</i>	44865	A	C	1260 (420)	GT <u>T</u> →GT <u>G</u>	Val→Val
	<i>caiT/fixA</i>	46353	T	C	−229/−245	–	–
	<i>fixB</i>	47721	C	A	339 (113)	ACC <u>C</u> →AC <u>A</u>	Thr→Thr
	<i>ftsL</i>	94003	G	T	168 (56)	AC <u>G</u> →AC <u>T</u>	Thr→Thr
	<i>murF</i>	98071	T	G	618 (206)	TT <u>T</u> →TT <u>G</u>	Phe→Leu
	<i>murD</i>	100218	A	C	328 (110)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>pdhR</i>	125265	A	C	370 (124)	<u>A</u> AG→ <u>C</u> AG	Lys→Gln
	<i>pdhR</i>	125630	T	G	735 (245)	TC <u>T</u> →TC <u>G</u>	Ser→Ser
	<i>acnB</i>	136422	A	C	2004 (668)	GC <u>A</u> →GC <u>C</u>	Ala→Ala
	<i>speE/yacC</i>	139321	T	G	−54/+52	–	–
	<i>yadG</i>	145773	A	C	192 (64)	GT <u>A</u> →GT <u>C</u>	Val→Val
	<i>yadK</i>	154388	A	C	53 (18)	C <u>T</u> A→C <u>G</u> A	Leu→Arg
	<i>htrE</i>	156770	A	C	1501 (501)	<u>T</u> CG→ <u>G</u> CG	Ser→Ala
	<i>pcnB</i>	161041	T	G	904 (302)	<u>A</u> AC→ <u>C</u> AC	Asn→His
	<i>yadR</i>	179508	T	G	57 (19)	GT <u>T</u> →GT <u>G</u>	Val→Val
	<i>pfs</i>	181491	T	G	505 (169)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>ldcC</i>	212537	T	G	17 (6)	A <u>T</u> T→A <u>G</u> T	Ile→Ser
	<i>ldcC</i>	214405	A	C	1885 (629)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>gmhB/rrsH</i>	226275	A	C	+30/−334	–	–
	<i>yafE/mltD</i>	235495	T	G	+22/+26	–	–
	<i>yhhI</i>	243156	A	C	344 (115)	C <u>A</u> T→C <u>C</u> T	His→Pro
	<i>fhiA†</i>	253813	A	C	818	–	–
	<i>ykgA</i>	289049	C	A	736 (246)	<u>G</u> GG→ <u>T</u> GG	Gly→Trp
	<i>ykgF</i>	295469	T	G	483 (161)	GAT→GAG	Asp→Glu

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20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>betA</i>	298983	T	G	914 (305)	C <u>A</u> A→C <u>C</u> A	Gln→Pro
	<i>betI</i>	301762	A	C	222 (74)	AA <u>T</u> →AA <u>G</u>	Asn→Lys
	<i>yahJ</i>	314518	A	C	745 (249)	<u>A</u> GT→ <u>C</u> GT	Ser→Arg
	<i>yahK</i>	315566	A	C	34 (12)	<u>A</u> AA→C <u>A</u> A	Lys→Gln
	<i>prpE</i>	325864	A	C	509 (170)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>cynR</i>	330492	T	G	755 (252)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>cynT</i>	331559	T	G	205 (69)	<u>T</u> CC→ <u>G</u> CC	Ser→Ala
	<i>cynX</i>	332612	T	G	65 (22)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>cynX</i>	332980	T	G	433 (145)	<u>T</u> GG→ <u>G</u> GG	Trp→Gly
	<i>mhpA</i>	342446	A	C	1280 (427)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>mhpC</i>	344430	T	G	651 (217)	GCT <u>T</u> →G <u>C</u> G	Ala→Ala
	<i>aroM/yaiE</i>	375815	G	T	+64/-8	–	–
	<i>yajF</i>	379334	T	G	768 (256)	AA <u>T</u> →AA <u>G</u>	Asn→Lys
	<i>araJ</i>	380188	A	C	717 (239)	TT <u>T</u> →TT <u>G</u>	Phe→Leu
	<i>sbcC</i>	383083	A	C	1094 (365)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>yajB/queA</i>	393379	G	T	-38/-55	–	–
	<i>secF</i>	398007	T	G	80 (27)	T <u>T</u> C→T <u>G</u> C	Phe→Cys
	<i>yajD/tsx</i>	399655	C	A	+281/+7	–	–
	<i>ribH</i>	403444	A	C	376 (126)	<u>A</u> TC→ <u>C</u> TC	Ile→Leu
	<i>yajO</i>	405910	C	A	648 (216)	CG <u>G</u> →CG <u>T</u>	Arg→Arg
	<i>thiI/thiJ</i>	411430	T	G	+11/+43	–	–
	<i>cyoE/cyoD</i>	418409	A	C	-1/+11	–	–
	<i>yajG</i>	424738	T	G	135 (45)	GT <u>A</u> →GT <u>C</u>	Val→Val
	<i>tig</i>	426998	C	A	1161 (387)	TT <u>C</u> →TT <u>A</u>	Phe→Leu
	<i>insL-2/lon</i>	430835	C	T	-48/-108	–	–
	<i>ybaV/ybaW</i>	436416	T	G	+53/-53	–	–
	<i>maa</i>	451789	A	G	197 (66)	T <u>T</u> T→T <u>C</u> T	Phe→Ser
	<i>kefA</i>	461180	A	C	2578 (860)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>apt</i>	463771	A	C	293 (98)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>aes</i>	471680	T	G	361 (121)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu

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	<i>gsk/ybaL</i>	473602	T	G	+106/+27	–	–
	<i>fsr</i>	475904	C	A	860 (287)	G <u>G</u> G→G <u>T</u> G	Gly→Val
	<i>ybbA</i>	492156	C	A	256 (86)	<u>C</u> G <u>C</u> → <u>A</u> G <u>C</u>	Arg→Ser
	<i>ybbP</i>	493795	T	G	1212 (404)	CT <u>T</u> →CT <u>G</u>	Leu→Leu
	<i>ybbP</i>	494682	T	G	2099 (700)	G <u>T</u> G→G <u>G</u> G	Val→Gly
	<i>ybbD/yibG</i>	501689	G	T	+622/+122	–	–
	<i>gcl</i>	506450	T	G	369 (123)	G <u>C</u> <u>T</u> →G <u>C</u> <u>G</u>	Ala→Ala
	<i>hyi</i>	508599	A	C	724 (242)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>allC</i>	516413	T	G	1046 (349)	T <u>A</u> C→T <u>C</u> C	Tyr→Ser
	<i>cysS</i>	527702	A	C	984 (328)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>sfmH</i>	535238	T	G	783 (261)	GAT <u>T</u> →GAG <u>G</u>	Asp→Glu
	<i>renD</i>	540028	A	C	77 (26)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>ybcM</i>	543686	T	G	343 (115)	<u>T</u> TA→ <u>G</u> TA	Leu→Val
	<i>nfrA</i>	565482	T	G	824 (275)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>cusS</i>	575344	A	C	330 (110)	TT <u>T</u> →TT <u>G</u>	Phe→Leu
	<i>cusR/cusC</i>	576388	A	C	–42/–115	–	–
	<i>cusA</i>	582155	A	C	2539 (847)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>entF</i>	597017	A	C	153 (51)	GG <u>A</u> →GG <u>C</u>	Gly→Gly
	<i>citT</i>	627805	T	G	827 (276)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>citA</i>	635217	A	C	395 (132)	G <u>A</u> G→G <u>C</u> G	Glu→Ala
	<i>ccrB</i>	640290	T	G	231 (77)	CT <u>A</u> →CT <u>C</u>	Leu→Leu
	<i>ybeF</i>	643694	T	G	268 (90)	<u>A</u> TG→ <u>C</u> TG	Met→Leu
	<i>mrdB</i>	648307	A	C	591 (197)	ATT <u>T</u> →AT <u>G</u>	Ile→Met
	<i>mrDA</i>	649391	T	A	1411 (471)	<u>A</u> TC→ <u>T</u> TC	Ile→Phe
	<i>ybeR</i>	659814	T	G	520 (174)	<u>T</u> CT→ <u>G</u> CT	Ser→Ala
	<i>glI</i>	668787	A	C	193 (65)	<u>T</u> AC→ <u>G</u> AC	Tyr→Asp
	<i>asnB/nagD</i>	681317	T	G	–45/+352	–	–
	<i>nagC</i>	683496	A	C	194 (65)	G <u>T</u> T→G <u>G</u> T	Val→Gly
	<i>glnS/ybfM</i>	690209	T	G	+357/–220	–	–
	<i>kdpB</i>	708381	T	G	751 (251)	<u>A</u> GC→ <u>C</u> GC	Ser→Arg

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20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>ybfO</i>	714041	A	C	78 (26)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>ybfO</i>	714992	A	C	1029 (343)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>phrB</i>	721536	T	G	1137 (379)	AA <u>T</u> →AA <u>G</u>	Asn→Lys
	<i>ybgJ</i>	724534	A	C	157 (53)	<u>A</u> TC→ <u>C</u> TC	Ile→Leu
	<i>ybgK</i>	725676	A	C	649 (217)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>abrB</i>	728010	T	G	545 (182)	A <u>A</u> T→A <u>C</u> T	Asn→Thr
	<i>ybgQ</i>	731342	T	G	1619 (540)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>ybgQ</i>	731494	T	G	1467 (489)	CC <u>A</u> →CC <u>C</u>	Pro→Pro
	<i>gltA/sdhC</i>	735533	T	G	−274/−435	–	–
	<i>sucB</i>	743093	G	A	781 (261)	<u>G</u> TC→ <u>A</u> TC	Val→Met
	<i>ybgG</i>	749853	A	C	994 (332)	<u>A</u> TC→ <u>C</u> TC	Ile→Leu
	<i>aroG</i>	766329	T	G	68 (23)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>ybhC</i>	786559	T	G	1259 (420)	AA <u>A</u> →A <u>C</u> A	Lys→Thr
	<i>ECB_00731/ECB_00732</i>	791065	A	C	+311/−390	–	–
	<i>ECB_00732</i>	791672	T	G	218 (73)	C <u>T</u> T→C <u>G</u> T	Leu→Arg
	<i>uvrB</i>	808158	T	G	2007 (669)	TT <u>T</u> →TT <u>G</u>	Phe→Leu
	<i>ybhO</i>	816306	T	G	59 (20)	T <u>A</u> T→T <u>C</u> T	Tyr→Ser
	<i>ybiB</i>	828820	T	G	948 (316)	GT <u>T</u> →GT <u>G</u>	Val→Val
	<i>ybiF</i>	842160	A	C	563 (188)	T <u>I</u> A→T <u>G</u> A	Leu→Stop
	<i>ybiR</i>	847206	T	G	935 (312)	T <u>I</u> A→T <u>G</u> A	Leu→Stop
	<i>ybiV</i>	852041	A	C	540 (180)	TT <u>T</u> →TT <u>G</u>	Phe→Leu
	<i>ybiY</i>	855272	A	C	819 (273)	CT <u>T</u> →CT <u>G</u>	Leu→Leu
	<i>yliG</i>	869618	T	G	970 (324)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>cmr</i>	876767	A	C	543 (181)	GC <u>A</u> →GC <u>C</u>	Ala→Ala
	<i>ECB_00828/ECB_00829</i>	889497	T	G	−78/−194	–	–
	<i>ECB_00833</i>	894179	T	G	571 (191)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>tfaE</i>	902709	T	G	378 (126)	GC <u>A</u> →GC <u>C</u>	Ala→Ala
	<i>rimK/ybjN</i>	909616	T	G	+40/−48	–	–
	<i>ybjN/potF</i>	910316	T	C	+176/−175	–	–
	<i>artM</i>	918143	A	C	99 (33)	TT <u>T</u> →TT <u>G</u>	Phe→Leu

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>ybjR</i>	921642	T	G	23 (8)	G <u>T</u> C→G <u>G</u> C	Val→Gly
	<i>ybjE</i>	931180	A	C	385 (129)	<u>T</u> TA→ <u>G</u> TA	Leu→Val
	<i>clpA</i>	940678	C	T	566 (189)	<u>C</u> C <u>G</u> →C <u>T</u> G	Pro→Leu
	<i>pflB</i>	969305	T	G	1343 (448)	A <u>A</u> C→A <u>C</u> C	Asn→Thr
	<i>pflB</i>	970635	T	G	13 (5)	<u>A</u> AT→ <u>C</u> AT	Asn→His
	<i>focA/ycaO</i>	971637	T	G	−78/+327	–	–
	<i>ycbB</i>	999454	A	C	1316 (439)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>ompF</i>	1003478	A	C	597 (199)	T <u>C</u> <u>T</u> →T <u>C</u> <u>G</u>	Ser→Ser
	<i>ompF/asnS</i>	1004193	C	A	−119/+483	–	–
	<i>ompF/asnS</i>	1004251	T	C	−177/+425	–	–
	<i>ycbS</i>	1016770	T	G	464 (155)	A <u>T</u> C→A <u>G</u> C	Ile→Ser
	<i>ycbS</i>	1016891	T	G	585 (195)	T <u>C</u> <u>T</u> →T <u>C</u> <u>G</u>	Ser→Ser
	<i>ompA</i>	1036240	T	G	905 (302)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>yccT/yccU</i>	1044908	A	C	−44/−129	–	–
	<i>yccY</i>	1061648	A	C	120 (40)	G <u>G</u> <u>T</u> →G <u>G</u> <u>G</u>	Gly→Gly
	<i>ycdI</i>	1086834	A	C	406 (136)	<u>T</u> GC→ <u>G</u> GC	Cys→Gly
	<i>ycdO</i>	1098758	A	C	231 (77)	G <u>A</u> <u>A</u> →G <u>A</u> <u>C</u>	Glu→Asp
	<i>ycdS</i>	1107001	G	T	1574 (525)	T <u>C</u> A→T <u>A</u> A	Ser→Stop
	<i>ycdS/ycdT</i>	1108953	T	G	−379/−208	–	–
	<i>csgD/csgB</i>	1118093	A	C	−328/−427	–	–
	<i>ymdC</i>	1121972	A	C	1049 (350)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>yceE</i>	1129502	T	G	558 (186)	T <u>T</u> <u>T</u> →T <u>T</u> <u>G</u>	Phe→Leu
	<i>pyrC</i>	1136897	T	G	283 (95)	<u>A</u> AC→ <u>C</u> AC	Asn→His
	<i>flgD</i>	1147072	T	G	647 (216)	C <u>T</u> C→C <u>G</u> C	Leu→Arg
	<i>acpP</i>	1166321	T	G	138 (46)	G <u>C</u> <u>T</u> →G <u>C</u> <u>G</u>	Ala→Ala
	<i>ycfH</i>	1171882	T	G	537 (179)	A <u>T</u> <u>T</u> →A <u>T</u> <u>G</u>	Ile→Met
	<i>fhuE</i>	1175474	T	G	647 (216)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>ycfS</i>	1184149	A	C	821 (274)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>ymgC</i>	1216957	A	C	49 (17)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>ycgG</i>	1218309	A	C	821 (274)	G <u>A</u> A→G <u>C</u> A	Glu→Ala

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>ycgH</i> †	1221358	T	G	1597	–	–
	<i>ycgM</i>	1228779	T	G	540 (180)	TTT→TTG	Phe→Leu
	<i>hlyE</i> †	1230389	C	A	156	–	–
	<i>mltE</i>	1243473	A	C	144 (48)	GCA→GCC	Ala→Ala
	<i>ycgR/ymgE</i>	1244728	A	C	–51/–150	–	–
	<i>dhaM</i>	1248380	A	C	885 (295)	GAT→GAG	Asp→Glu
	<i>dhaR</i>	1252845	T	G	1630 (544)	TAC→GAC	Tyr→Asp
	<i>narX</i>	1277282	T	G	487 (163)	ATG→CTG	Met→Leu
	<i>narK</i>	1278683	A	C	577 (193)	ATT→CTT	Ile→Leu
	<i>narI/ychS</i>	1286699	C	A	+23/–160	–	–
	<i>cls</i>	1305994	A	C	246 (82)	CTT→CTG	Leu→Leu
	<i>topA</i>	1329516	C	T	97 (33)	CAC→TAC	His→Tyr
	<i>topA</i>	1329567	T	G	148 (50)	TCT→GCT	Ser→Ala
	<i>pyrF</i>	1340917	A	C	626 (209)	CAG→CCG	Gln→Pro
	<i>yciT</i>	1342163	A	C	555 (185)	GAT→GAG	Asp→Glu
	<i>yciR</i>	1344571	T	G	542 (181)	AAT→ACT	Asn→Thr
	<i>yciR</i>	1344677	A	C	436 (146)	TTG→GTG	Leu→Val
	<i>yciW</i>	1347413	A	C	1064 (355)	CTG→CGG	Leu→Arg
	<i>sapA</i>	1355298	A	C	182 (61)	CTT→CGT	Leu→Arg
	<i>ycjL</i>	1359652	T	G	165 (55)	CTT→CTG	Leu→Leu
	<i>goaG</i>	1364853	T	G	936 (312)	AAT→AAG	Asn→Lys
	<i>ycjG</i>	1388180	T	G	882 (294)	AGT→AGG	Ser→Arg
	<i>ynaI</i>	1394215	A	C	77 (26)	ATG→AGG	Met→Arg
	<i>ogt</i>	1397197	A	C	210 (70)	TTT→TTG	Phe→Leu
	<i>abgR/ydaL</i>	1402836	A	C	+17/–313	–	–
	<i>recE</i>	1412217	A	C	2340 (780)	GTT→GTG	Val→Val
	<i>ydfQ</i>	1417576	T	G	47 (16)	CTG→CGG	Leu→Arg
	<i>trkG</i>	1419306	T	G	645 (215)	TGT→TGG	Cys→Trp
	<i>ynbB</i>	1447783	A	C	224 (75)	TAT→TCT	Tyr→Ser
	<i>hrpA</i>	1456048	A	C	3600 (1200)	CGA→CGC	Arg→Arg

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20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>hrpA/ydcF</i>	1456319	A	C	+25/−247	–	–
	<i>trg</i>	1463096	A	C	518 (173)	G <u>A</u> T→G <u>C</u> T	Asp→Ala
	<i>ydcI</i>	1464880	C	A	301 (101)	<u>G</u> A <u>T</u> → <u>T</u> A <u>T</u>	Asp→Tyr
	<i>tehA</i>	1470975	T	G	294 (98)	G <u>T</u> <u>T</u> →G <u>T</u> <u>G</u>	Val→Val
	<i>ydcP</i>	1477302	A	C	412 (138)	<u>A</u> <u>T</u> <u>T</u> → <u>C</u> <u>T</u> <u>T</u>	Ile→Leu
	<i>ansP/yncG</i>	1496171	A	C	−79/−188	–	–
	<i>narU</i>	1518024	A	C	188 (63)	A <u>T</u> C→A <u>G</u> C	Ile→Ser
	<i>yddL</i>	1520150	T	G	30 (10)	G <u>T</u> <u>A</u> →G <u>T</u> <u>C</u>	Val→Val
	<i>yddG</i>	1520715	A	C	548 (183)	<u>T</u> <u>I</u> <u>T</u> → <u>T</u> <u>G</u> <u>T</u>	Phe→Cys
	<i>fdnG</i>	1522293	A	C	743 (248)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>fdnH</i>	1525072	T	G	462 (154)	G <u>A</u> <u>T</u> →G <u>A</u> <u>G</u>	Asp→Glu
	<i>yddP</i>	1532839	A	C	117 (39)	G <u>G</u> <u>T</u> →G <u>G</u> <u>G</u>	Gly→Gly
	<i>yddW</i>	1541538	T	G	1237 (413)	<u>A</u> <u>C</u> <u>C</u> → <u>C</u> <u>C</u> <u>C</u>	Thr→Pro
	<i>gadB</i>	1544672	T	G	1325 (442)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>pqqL</i>	1547418	T	G	1736 (579)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>ydeN</i>	1555678	A	C	798 (266)	G <u>T</u> <u>T</u> →G <u>T</u> <u>G</u>	Val→Val
	<i>ydeU†</i>	1570553	A	C	80	–	–
	<i>yneE</i>	1574967	T	G	601 (201)	<u>A</u> <u>T</u> <u>T</u> → <u>C</u> <u>T</u> <u>T</u>	Ile→Leu
	<i>yneE</i>	1575413	A	C	155 (52)	<u>T</u> <u>I</u> <u>C</u> → <u>T</u> <u>G</u> <u>C</u>	Phe→Cys
	<i>eamA/ydeE</i>	1588943	T	G	−40/−155	–	–
	<i>ydfJ</i>	1599820	A	C	233 (78)	G <u>I</u> G→G <u>G</u> G	Val→Gly
	<i>ydfP</i>	1611621	T	G	101 (34)	G <u>A</u> T→G <u>C</u> T	Asp→Ala
	<i>speG</i>	1633778	A	C	400 (134)	<u>A</u> <u>T</u> <u>T</u> → <u>C</u> <u>T</u> <u>T</u>	Ile→Leu
	<i>ynfD</i>	1634870	T	G	111 (37)	A <u>T</u> <u>T</u> →A <u>T</u> <u>G</u>	Ile→Met
	<i>ynfI</i>	1642043	A	C	343 (115)	<u>A</u> <u>T</u> <u>T</u> → <u>C</u> <u>T</u> <u>T</u>	Ile→Leu
	<i>ynfM</i>	1647889	A	C	996 (332)	G <u>G</u> <u>A</u> →G <u>G</u> <u>C</u>	Gly→Gly
	<i>ydgD</i>	1649169	T	G	15 (5)	A <u>T</u> <u>T</u> →A <u>T</u> <u>G</u>	Ile→Met
	<i>rstA</i>	1659543	T	G	199 (67)	<u>I</u> G <u>T</u> → <u>G</u> G <u>T</u>	Cys→Gly
	<i>tus</i>	1661682	T	G	229 (77)	<u>T</u> <u>T</u> A→ <u>G</u> <u>T</u> A	Leu→Val
	<i>ydgK</i>	1682608	A	C	165 (55)	C <u>A</u> A→C <u>A</u> C	Gln→His

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>ydgR</i>	1690600	T	G	638 (213)	T <u>T</u> C→T <u>G</u> C	Phe→Cys
	<i>pdxY</i>	1692302	A	C	782 (261)	A <u>T</u> G→A <u>G</u> G	Met→Arg
	<i>lhr</i>	1706773	C	A	493 (165)	<u>C</u> GC→ <u>A</u> GC	Arg→Ser
	<i>lhr</i>	1707834	T	G	1554 (518)	G <u>C</u> T→G <u>C</u> G	Ala→Ala
	<i>lhr</i>	1708787	A	C	2507 (836)	T <u>A</u> T→T <u>C</u> T	Tyr→Ser
	<i>ydhO</i>	1712230	T	G	602 (201)	A <u>T</u> C→A <u>G</u> C	Ile→Ser
	<i>ECB_01628</i>	1714815	A	C	28 (10)	<u>T</u> TG→ <u>G</u> TG	Leu→Val
	<i>ydhY/ydhZ</i>	1732027	A	C	−283/+172	–	–
	<i>ydhZ/pykF</i>	1732450	A	C	−42/−515	–	–
	<i>sufD</i>	1738400	A	C	659 (220)	T <u>T</u> T→T <u>G</u> T	Phe→Cys
	<i>ydiP/ydiQ</i>	1756840	A	C	−271/−45	–	–
	<i>ydiS</i>	1759732	T	G	1070 (357)	G <u>T</u> T→G <u>G</u> T	Val→Gly
	<i>ydiD</i>	1761548	A	C	1304 (435)	G <u>A</u> T→G <u>C</u> T	Asp→Ala
	<i>ydiU</i>	1767478	T	G	1035 (345)	CT <u>A</u> →CT <u>C</u>	Leu→Leu
	<i>yniA</i>	1785895	T	G	835 (279)	<u>T</u> CA→ <u>G</u> CA	Ser→Ala
	<i>yniB/yniC</i>	1786512	T	G	−14/−133	–	–
	<i>ydjO</i>	1790190	T	G	220 (74)	<u>A</u> AG→ <u>C</u> AG	Lys→Gln
	<i>celF</i>	1794984	T	G	782 (261)	T <u>A</u> T→T <u>C</u> T	Tyr→Ser
	<i>celF</i>	1795074	T	G	692 (231)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>astE</i>	1803438	A	C	751 (251)	<u>I</u> AT→ <u>G</u> AT	Tyr→Asp
	<i>astD</i>	1806589	C	A	411 (137)	G <u>T</u> G→G <u>T</u> I	Val→Val
	<i>sppA</i>	1826639	T	G	575 (192)	T <u>I</u> G→T <u>G</u> G	Leu→Trp
	<i>pncA/ydjE</i>	1829798	A	C	+42/+51	–	–
	<i>ydjH</i>	1834004	A	C	154 (52)	<u>T</u> CT→ <u>G</u> CT	Ser→Ala
	<i>gapA</i>	1840904	A	C	905 (302)	A <u>A</u> C→A <u>C</u> C	Asn→Thr
	<i>yoaE</i>	1880102	A	C	63 (21)	AT <u>I</u> →AT <u>G</u>	Ile→Met
	<i>yobF/yebO</i>	1886192	A	C	−22/+647	–	–
	<i>yobF/yebO</i>	1886806	T	G	−636/+33	–	–
	<i>yebZ</i>	1902366	T	G	802 (268)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>yebZ</i>	1902841	A	C	327 (109)	CT <u>I</u> →CT <u>G</u>	Leu→Leu

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20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>yobB</i>	1904444	T	G	429 (143)	TT <u>I</u> →TT <u>G</u>	Phe→Leu
	<i>ptrB</i>	1905858	A	C	1558 (520)	<u>I</u> AT→ <u>G</u> AT	Tyr→Asp
	<i>yebF/yebG</i>	1909010	A	C	−32/+23	–	–
	<i>zwf</i>	1913540	A	C	1351 (451)	<u>I</u> CC→ <u>G</u> CC	Ser→Ala
	<i>znuC</i>	1921783	T	G	546 (182)	CAT <u>I</u> →CAG <u>G</u>	His→Gln
	<i>torY</i>	1936330	A	C	573 (191)	ACT <u>I</u> →AC <u>G</u>	Thr→Thr
	<i>torY</i>	1936718	T	G	185 (62)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>cheW/cheA</i>	1952123	T	G	−14/+7	–	–
	<i>yecR</i>	1966410	C	A	195 (65)	GG <u>C</u> →GG <u>A</u>	Gly→Gly
	<i>uvrC</i>	1972086	T	G	548 (183)	C <u>A</u> G→C <u>C</u> G	Gln→Pro
	<i>uvrY</i>	1972702	A	C	504 (168)	AG <u>T</u> →AG <u>G</u>	Ser→Arg
	<i>yedV</i>	1975162	T	G	990 (330)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>yedW/yedX</i>	1976879	T	G	−57/−76	–	–
	<i>yedX</i>	1977296	T	G	342 (114)	AA <u>T</u> →AA <u>G</u>	Asn→Lys
	<i>yeeJ</i>	1987874	T	G	5013 (1671)	AA <u>T</u> →AA <u>G</u>	Asn→Lys
	<i>amn</i>	1993134	A	C	75 (25)	GT <u>A</u> →GT <u>C</u>	Val→Val
	<i>cbl</i>	1998375	T	G	540 (180)	CA <u>A</u> →CA <u>C</u>	Gln→His
	<i>cbl/nac</i>	1998939	T	G	−25/+77	–	–
	<i>cobS</i>	2002639	A	C	584 (195)	T <u>I</u> A→T <u>G</u> A	Leu→Stop
	<i>yeeZ</i>	2018771	A	C	801 (267)	GAT <u>I</u> →GAG <u>G</u>	Asp→Glu
	<i>wzzB/ugd</i>	2028307	T	G	−1/+142	–	–
	<i>gmd</i>	2061256	T	G	264 (88)	GCA <u>A</u> →GCC <u>C</u>	Ala→Ala
	<i>dcd</i>	2075227	A	C	195 (65)	CT <u>I</u> →CT <u>G</u>	Leu→Leu
	<i>yegD</i>	2081023	A	C	164 (55)	GAT <u>I</u> →GCT <u>C</u>	Asp→Ala
	<i>yegI</i>	2082685	G	A	1481 (494)	GCT <u>I</u> →GTT	Ala→Val
	<i>yegI</i>	2083757	G	T	409 (137)	<u>C</u> AC→ <u>A</u> AC	His→Asn
	<i>yegL/yegM</i>	2086607	T	G	−1028/−517	–	–
	<i>gatB</i>	2127664	A	C	263 (88)	A <u>T</u> T→AG <u>T</u>	Ile→Ser
	<i>insB-15‡</i>	2129116	A	C	268 (90)	<u>A</u> TG→ <u>C</u> TG	Met→Leu
	<i>metG</i>	2145923	A	C	427 (143)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln

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20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>yehM</i>	2159082	A	C	2192 (731)	C <u>A</u> T→C <u>C</u> T	His→Pro
	<i>yehU</i>	2165584	G	T	258 (86)	GT <u>C</u> →GT <u>A</u>	Val→Val
	<i>yohI</i>	2181209	T	G	416 (139)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>cdd</i>	2183839	A	C	758 (253)	G <u>A</u> T→G <u>C</u> T	Asp→Ala
	<i>yeiH</i>	2201029	T	G	75 (25)	GT <u>T</u> →GT <u>G</u>	Val→Val
	<i>yeiH</i>	2201070	T	G	116 (39)	G <u>T</u> T→G <u>G</u> T	Val→Gly
	<i>yeiI/yeiJ</i>	2204048	T	G	+22/+34	–	–
	<i>rtn</i>	2222411	T	G	498 (166)	AA <u>T</u> →AA <u>G</u>	Asn→Lys
	<i>yejE</i>	2226691	T	G	231 (77)	GA <u>T</u> →GA <u>G</u>	Asp→Glu
	<i>yejE</i>	2226991	A	C	531 (177)	CA <u>A</u> →CA <u>C</u>	Gln→His
	<i>yejK</i>	2234717	T	G	419 (140)	T <u>A</u> T→T <u>C</u> T	Tyr→Ser
	<i>yejM/proL</i>	2237327	A	C	+3/–72	–	–
	<i>napA</i>	2251433	T	G	1310 (437)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>yojI</i>	2258360	A	C	360 (120)	AT <u>T</u> →AT <u>G</u>	Ile→Met
	<i>yojL</i>	2260637	A	C	1002 (334)	TT <u>T</u> →TT <u>G</u>	Phe→Leu
	<i>atoS</i>	2266908	A	C	166 (56)	<u>A</u> AC→ <u>C</u> AC	Asn→His
	<i>gyrA</i>	2285933	T	G	188 (63)	T <u>A</u> T→T <u>C</u> T	Tyr→Ser
	<i>yfaL</i>	2290343	T	G	527 (176)	T <u>A</u> T→T <u>C</u> T	Tyr→Ser
	<i>glpC</i>	2303128	T	G	900 (300)	CA <u>T</u> →CA <u>G</u>	His→Gln
	<i>arnT</i>	2318103	A	C	683 (228)	C <u>A</u> G→C <u>C</u> G	Gln→Pro
	<i>pmrD/menE</i>	2320133	A	C	–82/+28	–	–
	<i>yfbB</i>	2323662	A	C	444 (148)	TAT <u>T</u> →TAG <u>G</u>	Tyr→Stop
	<i>ECB_02200</i>	2333346	A	C	682 (228)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>nuoM</i>	2335296	T	G	1270 (424)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>nuoG</i>	2341199	T	G	2564 (855)	A <u>A</u> C→A <u>C</u> C	Asn→Thr
	<i>pta</i>	2360181	A	C	1843 (615)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>yfcC</i>	2361786	A	C	1114 (372)	<u>A</u> AT→ <u>C</u> AT	Asn→His
	<i>yfcE</i>	2363106	T	G	275 (92)	C <u>A</u> A→C <u>C</u> A	Gln→Pro
	<i>hisM</i>	2368152	T	G	674 (225)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>accD</i>	2377269	T	G	250 (84)	<u>A</u> AG→ <u>C</u> AG	Lys→Gln

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>yfcK</i>	2387080	A	C	1725 (575)	CC <u>A</u> →CC <u>C</u>	Pro→Pro
	<i>dsdX</i>	2412193	G	T	120 (40)	GT <u>G</u> →GT <u>T</u>	Val→Val
	<i>yfdE</i>	2422436	T	G	960 (320)	TT <u>A</u> →TT <u>C</u>	Leu→Phe
	<i>yfdW</i>	2426725	A	C	757 (253)	<u>T</u> GG→ <u>G</u> GG	Trp→Gly
	<i>ypdE</i>	2439538	T	G	235 (79)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>ypdG</i>	2441230	A	C	890 (297)	A <u>T</u> C→A <u>G</u> C	Ile→Ser
	<i>mntH/nupC</i>	2446990	T	G	−59/−277	–	–
	<i>crr</i>	2465005	A	C	446 (149)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>yfeU</i>	2474580	A	C	66 (22)	TC <u>A</u> →TC <u>C</u>	Ser→Ser
	<i>amiA</i>	2480430	A	C	560 (187)	C <u>A</u> A→C <u>C</u> A	Gln→Pro
	<i>eutC</i>	2484188	T	G	629 (210)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>eutH/eutG</i>	2488969	G	C	−133/+184	–	–
	<i>eutG</i>	2489436	A	C	905 (302)	T <u>T</u> G→T <u>G</u> G	Leu→Trp
	<i>eutT</i>	2494611	A	C	523 (175)	<u>I</u> CT→ <u>G</u> CT	Ser→Ala
	<i>maeB</i>	2497928	T	G	1278 (426)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>talA</i>	2499945	A	C	452 (151)	A <u>A</u> C→A <u>C</u> C	Asn→Thr
	<i>ypfG</i>	2503021	G	T	585 (195)	CT <u>C</u> →CT <u>A</u>	Leu→Leu
	<i>acrD</i>	2510826	T	G	2404 (802)	<u>T</u> TC→ <u>G</u> TC	Phe→Val
	<i>acrD/yffB</i>	2511681	T	G	+145/−394	–	–
	<i>gcvR</i>	2520902	T	G	169 (57)	<u>T</u> CA→ <u>G</u> CA	Ser→Ala
	<i>hyfE</i>	2527624	A	C	537 (179)	AT <u>A</u> →AT <u>C</u>	Ile→Ile
	<i>yfgC</i>	2537679	G	T	766 (256)	<u>G</u> CC→ <u>T</u> CC	Ala→Ser
	<i>uraA</i>	2540745	A	C	236 (79)	G <u>T</u> A→G <u>G</u> A	Val→Gly
	<i>hcaT</i>	2587662	A	C	884 (295)	G <u>T</u> G→G <u>G</u> G	Val→Gly
	<i>yphA</i>	2594015	A	C	43 (15)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>yphA</i>	2594074	A	C	102 (34)	GG <u>A</u> →GG <u>C</u>	Gly→Gly
	<i>yfhB</i>	2619549	A	C	431 (144)	G <u>T</u> C→G <u>G</u> C	Val→Gly
	<i>rncS</i>	2625494	T	G	62 (21)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>rseB</i>	2629329	G	T	919 (307)	<u>C</u> GC→ <u>A</u> GC	Arg→Ser
	<i>yfiF</i>	2639091	T	G	931 (311)	<u>A</u> AC→ <u>C</u> AC	Asn→His

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>yfiQ</i>	2641700	T	G	255 (85)	CT <u>T</u> →CT <u>G</u>	Leu→Leu
	<i>yfiO</i>	2657995	T	C	442 (148)	<u>T</u> CC→ <u>C</u> CC	Ser→Pro
	<i>grpE</i>	2671458	A	C	546 (182)	GG <u>T</u> →GG <u>G</u>	Gly→Gly
	<i>ECB_02509</i>	2677546	A	C	130 (44)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>emrR</i>	2705690	A	C	62 (21)	G <u>A</u> T→G <u>C</u> T	Asp→Ala
	<i>hypF</i>	2732014	C	A	272 (91)	<u>C</u> GC→ <u>C</u> TC	Arg→Leu
	<i>hycE</i>	2740772	A	C	560 (187)	<u>C</u> TG→ <u>C</u> GG	Leu→Arg
	<i>hycA</i>	2745079	T	G	217 (73)	<u>A</u> AC→ <u>C</u> AC	Asn→His
	<i>pphB</i>	2754688	T	G	69 (23)	AT <u>T</u> →AT <u>G</u>	Ile→Met
	<i>ygbM</i>	2759524	A	C	428 (143)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>ygcQ</i>	2784272	A	C	8 (3)	A <u>T</u> C→A <u>G</u> C	Ile→Ser
	<i>ygcE</i>	2790674	T	G	279 (93)	GAT→GAG	Asp→Glu
	<i>ygcG</i>	2795341	T	G	703 (235)	<u>T</u> TT→ <u>G</u> TT	Phe→Val
	<i>chpA</i>	2799764	T	G	256 (86)	<u>A</u> GA→ <u>C</u> GA	Arg→Arg
	<i>barA</i>	2804425	T	G	441 (147)	AG <u>T</u> →AG <u>G</u>	Ser→Arg
	<i>barA</i>	2804511	A	C	527 (176)	G <u>A</u> G→G <u>C</u> G	Glu→Ala
	<i>gudX</i>	2809503	T	G	173 (58)	G <u>A</u> G→G <u>C</u> G	Glu→Ala
	<i>yqcB</i>	2812394	T	G	320 (107)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>sdaC</i>	2817492	T	G	335 (112)	A <u>T</u> C→A <u>G</u> C	Ile→Ser
	<i>ECB_02649</i>	2825941	A	C	278 (93)	<u>C</u> TG→ <u>C</u> GG	Leu→Arg
	<i>fucK</i>	2832497	A	C	441 (147)	TT <u>A</u> →TT <u>C</u>	Leu→Phe
	<i>ygdI</i>	2837626	T	G	139 (47)	<u>A</u> CC→ <u>C</u> CC	Thr→Pro
	<i>ygdL</i>	2840075	A	C	387 (129)	AT <u>T</u> →AT <u>G</u>	Ile→Met
	<i>amiC/argA</i>	2843663	A	C	−34/−198	–	–
	<i>ptr</i>	2851294	A	C	2210 (737)	G <u>T</u> A→G <u>G</u> A	Val→Gly
	<i>recC</i>	2853864	A	C	3184 (1062)	<u>T</u> CC→ <u>G</u> CC	Ser→Ala
	<i>ppdB</i>	2858322	T	G	14 (5)	G <u>A</u> G→G <u>C</u> G	Glu→Ala
	<i>mutH</i>	2864911	C	A	630 (210)	GG <u>C</u> →GG <u>A</u>	Gly→Gly
	<i>ygeD</i>	2868116	T	G	367 (123)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>aas</i>	2870517	T	G	118 (40)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>galR</i>	2872152	T	G	933 (311)	CCT <u>I</u> →CC <u>G</u>	Pro→Pro
	<i>yqeA</i>	2897800	T	G	585 (195)	ATT <u>I</u> →AT <u>G</u>	Ile→Met
	<i>xdhD/ygfO</i>	2909993	T	G	+53/-112	–	–
	<i>ygfS</i>	2914384	G	T	227 (76)	G <u>C</u> C→G <u>A</u> C	Ala→Asp
	<i>prfB</i>	2920951	T	G	1085 (362)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>dsbC</i>	2924475	A	C	101 (34)	A <u>T</u> C→A <u>G</u> C	Ile→Ser
	<i>ygfY</i>	2926721	A	C	103 (35)	<u>T</u> TA→ <u>G</u> TA	Leu→Val
	<i>visC</i>	2937857	A	C	212 (71)	C <u>T</u> C→C <u>G</u> C	Leu→Arg
	<i>sbm</i>	2947175	A	C	575 (192)	G <u>A</u> G→G <u>C</u> G	Glu→Ala
	<i>sbm</i>	2948477	A	C	1877 (626)	G <u>A</u> G→G <u>C</u> G	Glu→Ala
	<i>yggB</i>	2955191	A	C	368 (123)	T <u>T</u> A→T <u>G</u> A	Leu→Stop
	<i>mutY</i>	2988792	T	G	119 (40)	T <u>T</u> G→T <u>G</u> G	Leu→Trp
	<i>yafZ</i>	3006683	A	C	693 (231)	GG <u>A</u> →GG <u>C</u>	Gly→Gly
	<i>ECB_02822/insB-22</i>	3023893	T	G	-171/+67	–	–
	<i>ECB_02827</i>	3027011	T	G	660 (220)	TA <u>A</u> →TA <u>C</u>	Stop→Tyr
	<i>ECB_02827/ECB_02828</i>	3027683	A	C	-13/+2	–	–
	<i>yghG</i>	3040080	A	C	332 (111)	A <u>T</u> A→A <u>G</u> A	Ile→Arg
	<i>pppA/yghJ</i>	3041354	C	A	-68/+96	–	–
	<i>yghJ</i>	3043941	T	G	2063 (688)	G <u>A</u> T→G <u>C</u> T	Asp→Ala
	<i>yghJ</i>	3045069	G	T	935 (312)	A <u>C</u> C→A <u>A</u> C	Thr→Asn
	<i>ECB_02851</i>	3057690	A	C	246 (82)	GA <u>A</u> →GA <u>C</u>	Glu→Asp
	<i>yghA</i>	3084190	T	G	357 (119)	GT <u>T</u> →GT <u>G</u>	Val→Val
	<i>yqhD</i>	3089831	A	C	305 (102)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>ygiQ</i>	3093117	T	G	2202 (734)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>ygiW</i>	3103672	G	A	177 (59)	ACC <u>C</u> →ACT <u>I</u>	Thr→Thr
	<i>parE</i>	3109306	T	G	263 (88)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>ygiC</i>	3114886	T	G	294 (98)	TA <u>T</u> →TA <u>G</u>	Tyr→Stop
	<i>ygiE</i>	3116928	A	C	207 (69)	GG <u>A</u> →GG <u>C</u>	Gly→Gly
	<i>yqiG</i>	3121407	A	C	1175 (392)	G <u>A</u> T→G <u>C</u> T	Asp→Ala
	<i>yqiI</i>	3123757	A	C	290 (97)	T <u>A</u> T→T <u>C</u> T	Tyr→Ser

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>ygiF</i>	3133535	T	G	265 (89)	<u>A</u> GC→ <u>C</u> GC	Ser→Arg
	<i>ygiP</i>	3138764	A	C	310 (104)	<u>T</u> TT→ <u>G</u> TT	Phe→Val
	<i>ebgR</i>	3155168	G	A	886 (296)	<u>G</u> GC→ <u>A</u> GC	Gly→Ser
	<i>ygiK</i>	3162213	A	C	509 (170)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>ygiU</i>	3173835	T	G	858 (286)	GAT <u>T</u> →GAG <u>G</u>	Asp→Glu
	<i>exuT</i>	3178868	A	C	731 (244)	A <u>A</u> T→A <u>C</u> T	Asn→Thr
	<i>yqiG</i>	3184072	T	G	15 (5)	AT <u>T</u> →AT <u>G</u>	Ile→Met
	<i>yhaJ/yhaK</i>	3187264	A	C	−17/−88	–	–
	<i>yhaK</i>	3188042	T	G	691 (231)	<u>T</u> TG→ <u>G</u> TG	Leu→Val
	<i>tdcG</i>	3192570	T	G	114 (38)	TT <u>A</u> →TT <u>C</u>	Leu→Phe
	<i>agaR</i>	3211263	T	G	331 (111)	<u>A</u> TC→ <u>C</u> TC	Ile→Leu
	<i>agaS</i>	3217222	A	C	14 (5)	T <u>A</u> C→T <u>C</u> C	Tyr→Ser
	<i>agaY</i>	3219202	A	C	827 (276)	A <u>A</u> T→A <u>C</u> T	Asn→Thr
	<i>yraK</i>	3227037	T	G	463 (155)	<u>T</u> AT→ <u>G</u> AT	Tyr→Asp
	<i>yhbU</i>	3236743	A	C	25 (9)	<u>A</u> AT→ <u>C</u> AT	Asn→His
	<i>nlpI</i>	3243698	A	C	461 (154)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>infB</i>	3248957	A	T	2292 (764)	GAT <u>T</u> →GA <u>A</u>	Asp→Glu
	<i>nusA/yhbC</i>	3252768	T	G	−8/+20	–	–
	<i>yhbC/metZ</i>	3253256	A	C	−16/+191	–	–
	<i>hflB</i>	3260604	T	G	1566 (522)	GA <u>A</u> →GA <u>C</u>	Glu→Asp
	<i>rrmJ</i>	3262857	A	C	42 (14)	CT <u>T</u> →CT <u>G</u>	Leu→Leu
	<i>yrbA/yrbB</i>	3272110	A	C	−72/+88	–	–
	<i>yhbH</i>	3281577	A	C	170 (57)	A <u>A</u> C→A <u>C</u> C	Asn→Thr
	<i>arcB</i>	3288092	G	A	169 (57)	<u>C</u> GT→ <u>T</u> GT	Arg→Cys
	<i>nanK</i>	3297875	A	C	374 (125)	G <u>T</u> C→G <u>G</u> C	Val→Gly
	<i>dcuD</i>	3303212	T	G	446 (149)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>dcuD</i>	3303353	A	C	587 (196)	T <u>A</u> C→T <u>C</u> C	Tyr→Ser
	<i>maf</i>	3325845	T	G	425 (142)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>yhdH/accB</i>	3333231	T	G	+882/−96	–	–
	<i>yhdG/fis</i>	3339158	A	C	+22/−4	–	–

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>acrE</i>	3341864	C	A	110 (37)	C <u>C</u> G→C <u>A</u> G	Pro→Gln
	<i>smg</i>	3360344	A	C	26 (9)	T <u>I</u> T→T <u>G</u> T	Phe→Cys
	<i>rpsD</i>	3369432	T	C	149 (50)	G <u>A</u> C→G <u>G</u> C	Asp→Gly
	<i>rpsM</i>	3370027	T	A	350 (117)	A <u>A</u> G→A <u>T</u> G	Lys→Met
	<i>rplF</i>	3373799	A	C	247 (83)	T <u>T</u> C→ <u>G</u> T <u>C</u>	Phe→Val
	<i>gspC</i>	3384112	T	G	630 (210)	GAT <u>I</u> →GAG <u>G</u>	Asp→Glu
	<i>fkpA</i>	3404580	T	G	745 (249)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>yhjK</i>	3414905	T	G	210 (70)	GGT <u>I</u> →GGG <u>G</u>	Gly→Gly
	<i>yhjV</i>	3436635	T	G	638 (213)	A <u>A</u> G→A <u>C</u> G	Lys→Thr
	<i>hslO</i>	3459043	A	C	586 (196)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>pckA</i>	3462285	A	C	784 (262)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>yhgF</i>	3466075	A	C	4 (2)	<u>A</u> AT→ <u>C</u> AT	Asn→His
	<i>feoB</i>	3470129	T	G	1058 (353)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>yhgl/gntT</i>	3475086	T	G	+222/−139	–	–
	<i>malT</i>	3483047	C	A	1363 (455)	<u>C</u> GC→ <u>A</u> GC	Arg→Ser
	<i>rtcA</i>	3485307	A	C	143 (48)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>glpE</i>	3490112	C	T	313 (105)	<u>G</u> CG→ <u>A</u> CG	Ala→Thr
	<i>ECB_03279</i>	3493524	T	G	406 (136)	<u>A</u> CC→ <u>C</u> CC	Thr→Pro
	<i>glgP</i>	3495915	G	T	2121 (707)	GG <u>C</u> →GGA <u>A</u>	Gly→Gly
	<i>glgA</i>	3498495	A	C	993 (331)	GAT <u>I</u> →GAG <u>G</u>	Asp→Glu
	<i>glgA</i>	3499025	T	G	463 (155)	<u>A</u> AG→ <u>C</u> AG	Lys→Gln
	<i>glgX</i>	3500952	A	C	1822 (608)	T <u>T</u> G→ <u>G</u> TG	Leu→Val
	<i>asd/yhgN</i>	3506469	A	C	−137/−55	–	–
	<i>yhgN</i>	3506840	T	G	317 (106)	T <u>I</u> T→T <u>G</u> T	Phe→Cys
	<i>gntU</i>	3507177	A	C	1338 (446)	AGT <u>I</u> →AGG <u>G</u>	Ser→Arg
	<i>ugpC</i>	3519804	T	G	53 (18)	C <u>A</u> G→C <u>C</u> G	Gln→Pro
	<i>ugpA</i>	3521505	A	C	83 (28)	T <u>I</u> T→T <u>G</u> T	Phe→Cys
	<i>livK</i>	3527310	A	C	925 (309)	<u>I</u> CT→ <u>G</u> CT	Ser→Ala
	<i>livK/yhhK</i>	3528587	A	C	−353/−71	–	–
	<i>zntA</i>	3537761	T	G	623 (208)	C <u>T</u> G→C <u>G</u> G	Leu→Arg

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>yhhS</i>	3541981	A	C	441 (147)	CAT <u>I</u> →CAG <u>G</u>	His→Gln
	<i>yhhS/yhhT</i>	3542534	A	C	−113/−19	–	–
	<i>yhhT</i>	3543278	A	C	726 (242)	GT <u>A</u> →GT <u>C</u>	Val→Val
	<i>acpT</i>	3543935	A	C	279 (93)	GA <u>A</u> →GAC <u>C</u>	Glu→Asp
	<i>yhhI</i>	3555146	G	T	81 (27)	TC <u>G</u> →TCT <u>I</u>	Ser→Ser
	<i>yhhJ</i>	3557298	C	A	194 (65)	C <u>G</u> G→CT <u>I</u> G	Arg→Leu
	<i>yhiI</i>	3561248	A	C	43 (15)	<u>T</u> TA→ <u>G</u> TA	Leu→Val
	<i>yhiM/yhiN</i>	3566725	T	G	+143/+172	–	–
	<i>prlC</i>	3574505	C	A	1368 (456)	CC <u>G</u> →CCT <u>I</u>	Pro→Pro
	<i>prlC</i>	3575750	T	G	123 (41)	GT <u>A</u> →GT <u>C</u>	Val→Val
	<i>gor</i>	3576996	A	C	8 (3)	A <u>A</u> A→AC <u>C</u> A	Lys→Thr
	<i>arsC/yhiS</i>	3581373	A	C	+21/−608	–	–
	<i>gadA</i>	3596144	T	G	928 (310)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>treF</i>	3599293	A	C	210 (70)	AA <u>A</u> →AAC <u>C</u>	Lys→Asn
	<i>yhjE/yhjG</i>	3605645	T	G	+45/+137	–	–
	<i>kdgK/yhjJ</i>	3609865	T	G	+25/+71	–	–
	<i>dctA/yhjK</i>	3612954	T	G	−15/+168	–	–
	<i>dctA/yhjK</i>	3613023	A	C	−84/+99	–	–
	<i>bcsC</i>	3617683	T	G	983 (328)	A <u>A</u> A→AC <u>C</u> A	Lys→Thr
	<i>bcsC</i>	3617837	A	C	829 (277)	<u>T</u> TA→ <u>G</u> TA	Leu→Val
	<i>yhjX</i>	3641648	A	C	818 (273)	C <u>I</u> G→C <u>G</u> G	Leu→Arg
	<i>bisC</i>	3644741	T	G	2058 (686)	GG <u>A</u> →GG <u>C</u>	Gly→Gly
	<i>cspA</i>	3650528	A	C	22 (8)	<u>A</u> TC→ <u>C</u> TC	Ile→Leu
	<i>yiaA</i>	3657614	A	C	206 (69)	A <u>T</u> A→A <u>G</u> A	Ile→Arg
	<i>malS</i>	3668060	T	G	108 (36)	TT <u>I</u> →TT <u>G</u>	Phe→Leu
	<i>ECB_03437</i>	3683249	T	G	940 (314)	<u>T</u> AC→ <u>G</u> AC	Tyr→Asp
	<i>ECB_03438</i>	3685258	A	C	1538 (513)	T <u>A</u> T→T <u>C</u> T	Tyr→Ser
	<i>yiaY/selB</i>	3692673	T	G	−153/+37	–	–
	<i>rhsA</i>	3699461	A	C	2585 (862)	A <u>A</u> C→A <u>C</u> C	Asn→Thr
	<i>yibL/ECB_03459</i>	3711747	A	C	+27/−517	–	–

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>ECB_03459</i>	3712483	A	C	220 (74)	<u>A</u> AG→ <u>C</u> AG	Lys→Gln
	<i>ECB_03460</i>	3713828	G	T	838 (280)	<u>G</u> GA→ <u>T</u> GA	Gly→Stop
	<i>ECB_03460</i>	3716330	A	C	3340 (1114)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>ECB_03460</i>	3716676	C	A	3686 (1229)	G <u>C</u> T→G <u>A</u> T	Ala→Asp
	<i>lldP</i>	3718474	T	G	266 (89)	T <u>T</u> C→T <u>G</u> C	Phe→Cys
	<i>lldP</i>	3718728	G	T	520 (174)	<u>G</u> GC→ <u>T</u> GC	Gly→Cys
	<i>cysE</i>	3722953	G	T	376 (126)	<u>C</u> GC→ <u>A</u> GC	Arg→Ser
	<i>secB</i>	3724516	T	G	379 (127)	<u>A</u> AC→ <u>C</u> AC	Asn→His
	<i>waaL</i>	3737701	T	G	126 (42)	GG <u>A</u> →GG <u>C</u>	Gly→Gly
	<i>waaO</i>	3743455	T	G	43 (15)	<u>A</u> CA→ <u>C</u> CA	Thr→Pro
	<i>coaD</i>	3748382	T	G	203 (68)	G <u>T</u> C→G <u>G</u> C	Val→Gly
	<i>dinD/yicG</i>	3757158	A	C	+218/-73	–	–
	<i>yicF</i>	3759405	T	G	123 (41)	AT <u>A</u> →AT <u>C</u>	Ile→Ile
	<i>spoT</i>	3762741	A	T	1985 (662)	A <u>A</u> A→A <u>I</u> A	Lys→Ile
	<i>ECB_03520</i>	3781098	T	G	1130 (377)	A <u>T</u> G→A <u>G</u> G	Met→Arg
	<i>ECB_03521</i>	3781398	A	C	63 (21)	CT <u>A</u> →CT <u>C</u>	Leu→Leu
	<i>ECB_03532</i>	3792298	A	C	404 (135)	C <u>A</u> T→C <u>C</u> T	His→Pro
	<i>yicK</i>	3797813	T	G	929 (310)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>ade</i>	3805135	A	C	1240 (414)	<u>A</u> TC→ <u>C</u> TC	Ile→Leu
	<i>ade</i>	3805357	A	C	1462 (488)	<u>A</u> TG→ <u>C</u> TG	Met→Leu
	<i>uhpT</i>	3806072	A	C	1028 (343)	G <u>T</u> G→G <u>G</u> G	Val→Gly
	<i>uhpT</i>	3806477	A	C	623 (208)	A <u>T</u> C→A <u>G</u> C	Ile→Ser
	<i>glvG</i>	3822363	A	C	65 (22)	A <u>T</u> G→A <u>G</u> G	Met→Arg
	<i>glvBC</i>	3822763	T	G	1281 (427)	TC <u>A</u> →TC <u>C</u>	Ser→Ser
	<i>glvBC</i>	3822784	A	C	1260 (420)	GAT <u>I</u> →GAG <u>G</u>	Asp→Glu
	<i>glvBC</i>	3824042	A	C	2 (1)	A <u>T</u> G→A <u>G</u> G	Met→Arg
	<i>dgoK</i>	3834332	T	G	504 (168)	TT <u>A</u> →TT <u>C</u>	Leu→Phe
	<i>dgoR</i>	3835234	A	C	288 (96)	AG <u>T</u> →AG <u>G</u>	Ser→Arg
	<i>dnaA</i>	3842724	T	G	1367 (456)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>yieG</i>	3856914	A	C	57 (19)	TT <u>T</u> →TT <u>G</u>	Phe→Leu

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>yieH</i>	3857359	T	G	223 (75)	<u>T</u> AC→ <u>G</u> AC	Tyr→Asp
	<i>yieH/yieI</i>	3857817	T	G	+15/-52	–	–
	<i>yieL</i>	3859873	T	G	1101 (367)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>bglF</i>	3865067	A	C	899 (300)	T <u>T</u> C→T <u>G</u> C	Phe→Cys
	<i>gidA/mioC</i>	3886222	A	C	-228/+151	–	–
	<i>yifB</i>	3911660	A	C	140 (47)	T <u>T</u> A→T <u>G</u> A	Leu→Stop
	<i>ilvA</i>	3917503	T	G	371 (124)	T <u>T</u> C→T <u>G</u> C	Phe→Cys
	<i>yifN†</i>	3922213	A	C	50	–	–
	<i>rep</i>	3923571	A	C	1093 (365)	<u>A</u> AC→ <u>C</u> AC	Asn→His
	<i>rfe</i>	3930609	T	G	894 (298)	AT <u>T</u> →AT <u>G</u>	Ile→Met
	<i>wecB</i>	3932175	T	G	243 (81)	AT <u>T</u> →AT <u>G</u>	Ile→Met
	<i>rffG</i>	3934468	A	C	147 (49)	CA <u>A</u> →CA <u>C</u>	Gln→His
	<i>wecD</i>	3936513	A	C	118 (40)	<u>A</u> GC→ <u>C</u> GC	Ser→Arg
	<i>wecG</i>	3942051	A	C	296 (99)	G <u>A</u> G→G <u>C</u> G	Glu→Ala
	<i>aslB</i>	3945610	T	G	853 (285)	<u>T</u> CG→ <u>G</u> CG	Ser→Ala
	<i>aslB</i>	3945896	A	C	1139 (380)	<u>C</u> <u>A</u> T→ <u>C</u> <u>C</u> T	His→Pro
	<i>cyaA/cyaY</i>	3955552	T	G	+23/+17	–	–
	<i>ECB_03690</i>	3962760	T	G	180 (60)	GA <u>A</u> →GA <u>C</u>	Glu→Asp
	<i>yigE/corA</i>	3963863	T	G	-77/-293	–	–
	<i>yigF</i>	3966983	T	G	252 (84)	CT <u>A</u> →CT <u>C</u>	Leu→Leu
	<i>rarD</i>	3968103	T	G	511 (171)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>rarD</i>	3968307	T	G	307 (103)	<u>A</u> GC→ <u>C</u> GC	Ser→Arg
	<i>yigI/pldA</i>	3969200	T	G	-68/-97	–	–
	<i>recQ</i>	3970954	A	C	670 (224)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>metR</i>	3976831	T	G	413 (138)	G <u>A</u> G→G <u>C</u> G	Glu→Ala
	<i>ECB_03713</i>	3984539	G	T	350 (117)	<u>C</u> <u>C</u> G→ <u>C</u> <u>A</u> G	Pro→Gln
	<i>ECB_03720</i>	3992033	A	C	128 (43)	<u>T</u> <u>T</u> T→T <u>G</u> T	Phe→Cys
	<i>ECB_03721</i>	3992619	T	G	530 (177)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>ECB_03723</i>	3994424	T	G	373 (125)	<u>A</u> TG→ <u>C</u> TG	Met→Leu
	<i>yigN</i>	3995913	A	C	110 (37)	G <u>A</u> A→G <u>C</u> A	Glu→Ala

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>ubiB</i>	3998776	A	C	80 (27)	A <u>A</u> →A <u>C</u> A	Lys→Thr
	<i>ubiB</i>	3998959	A	C	263 (88)	G <u>A</u> T→G <u>C</u> T	Asp→Ala
	<i>yigZ</i>	4011464	A	C	600 (200)	TT <u>A</u> →TT <u>C</u>	Leu→Phe
	<i>hemG/rrsA</i>	4013609	A	C	+83/−295	–	–
	<i>polA</i>	4027844	T	G	2582 (861)	A <u>T</u> T→A <u>G</u> T	Ile→Ser
	<i>yihA/yihI</i>	4029063	A	C	−37/−581	–	–
	<i>bipA</i>	4038134	T	G	1470 (490)	TC <u>T</u> →TC <u>G</u>	Ser→Ser
	<i>yihL</i>	4039222	A	C	518 (173)	A <u>A</u> →A <u>C</u> A	Lys→Thr
	<i>fdhE</i>	4058501	T	G	875 (292)	G <u>A</u> →G <u>C</u> A	Glu→Ala
	<i>fdhD</i>	4064362	T	G	200 (67)	C <u>T</u> C→C <u>G</u> C	Leu→Arg
	<i>frvB</i>	4070289	A	C	225 (75)	GAT <u>T</u> →GAG <u>G</u>	Asp→Glu
	<i>frvA</i>	4070711	T	G	260 (87)	A <u>A</u> →A <u>C</u> A	Lys→Thr
	<i>frvA</i>	4070746	T	G	225 (75)	GT <u>A</u> →GT <u>C</u>	Val→Val
	<i>rhaD</i>	4073894	T	G	137 (46)	A <u>A</u> →A <u>C</u> A	Lys→Thr
	<i>rhaD</i>	4073906	A	C	125 (42)	A <u>T</u> C→A <u>G</u> C	Ile→Ser
	<i>rhaA</i>	4075029	T	G	442 (148)	<u>A</u> CG→ <u>C</u> CG	Thr→Pro
	<i>rhaR</i>	4078976	A	C	939 (313)	TA <u>A</u> →TAC <u>C</u>	Stop→Tyr
	<i>sodA/kdgT</i>	4081115	A	C	+203/−57	–	–
	<i>cdh</i>	4089472	A	C	61 (21)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>cdh</i>	4089879	A	C	468 (156)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>glpF</i>	4096930	A	C	643 (215)	<u>T</u> GG→ <u>G</u> GG	Trp→Gly
	<i>hslU</i>	4100655	C	T	576 (192)	AT <u>G</u> →AT <u>A</u>	Met→Ile
	<i>ftsN</i>	4102718	A	C	105 (35)	GCT <u>T</u> →GCG <u>G</u>	Ala→Ala
	<i>yijE</i>	4116720	T	G	506 (169)	A <u>T</u> T→A <u>G</u> T	Ile→Ser
	<i>gldA</i>	4118298	T	G	845 (282)	C <u>A</u> G→C <u>C</u> G	Gln→Pro
	<i>ptsA</i>	4121449	T	G	880 (294)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>udhA</i>	4139889	T	G	763 (255)	<u>A</u> AG→ <u>C</u> AG	Lys→Gln
	<i>trmA</i>	4142501	T	G	631 (211)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>tuf/secE</i>	4156805	T	G	+66/−164	–	–
	<i>rpoB</i>	4163173	T	G	2318 (773)	C <u>T</u> G→C <u>G</u> G	Leu→Arg

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>rpoC</i>	4166668	A	C	1708 (570)	<u>A</u> AA→C <u>A</u> A	Lys→Gln
	<i>thiC</i>	4175612	A	C	99 (33)	AT <u>T</u> →AT <u>G</u>	Ile→Met
	<i>hupA</i>	4180045	A	C	153 (51)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>yjaH</i>	4180803	A	C	626 (209)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>iclR</i>	4201911	C	T	649 (217)	<u>G</u> CG→ <u>A</u> CG	Ala→Thr
	<i>metH</i>	4202859	A	C	101 (34)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>malE</i>	4225047	A	C	305 (102)	T <u>T</u> G→T <u>G</u> G	Leu→Trp
	<i>lamB</i>	4227464	T	G	562 (188)	<u>T</u> AT→ <u>G</u> AT	Tyr→Asp
	<i>yjbI</i>	4230064	T	G	178 (60)	<u>T</u> GC→ <u>G</u> GC	Cys→Gly
	<i>yjbN</i>	4240745	T	G	145 (49)	<u>T</u> CC→ <u>G</u> CC	Ser→Ala
	<i>aphA</i>	4248845	T	G	500 (167)	T <u>T</u> T→T <u>G</u> T	Phe→Cys
	<i>actP</i>	4262744	A	C	1092 (364)	GCT→G <u>C</u> <u>G</u>	Ala→Ala
	<i>nrfG</i>	4272929	T	G	454 (152)	<u>T</u> CC→ <u>G</u> CC	Ser→Ala
	<i>yjcR/yjcS</i>	4283162	A	C	−231/+272	–	–
	<i>rpiR/rpiB</i>	4292123	A	C	−310/−49	–	–
	<i>phnK</i>	4296969	T	G	618 (206)	TT <u>A</u> →TT <u>C</u>	Leu→Phe
	<i>basS</i>	4311978	A	C	9 (3)	TT <u>T</u> →TT <u>G</u>	Phe→Leu
	<i>lysU</i>	4332868	A	C	570 (190)	AT <u>T</u> →AT <u>G</u>	Ile→Met
	<i>groEL</i>	4350624	A	C	879 (293)	GC <u>A</u> →GC <u>C</u>	Ala→Ala
	<i>yjeK</i>	4353840	A	C	529 (177)	<u>T</u> GG→ <u>G</u> GG	Trp→Gly
	<i>yjfI</i>	4388311	A	C	24 (8)	CT <u>A</u> →CT <u>C</u>	Leu→Leu
	<i>yjfC</i>	4391658	T	G	476 (159)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>aidB</i>	4392506	C	A	77 (26)	G <u>C</u> G→G <u>A</u> G	Ala→Glu
	<i>rpsF</i>	4403583	A	C	311 (104)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>rplI/ECB_04071</i>	4404811	T	G	+99/+3	–	–
	<i>ECB_04071</i>	4405984	A	C	108 (36)	AT <u>T</u> →AT <u>G</u>	Ile→Met
	<i>ECB_04076</i>	4412016	T	G	218 (73)	C <u>A</u> T→C <u>C</u> T	His→Pro
	<i>fklB</i>	4414202	A	C	269 (90)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>ytfL/msrA</i>	4425276	T	G	−304/+19	–	–
	<i>ytfR</i>	4435204	T	G	897 (299)	CG <u>T</u> →CG <u>G</u>	Arg→Arg

Supplementary Table 3. Details of SNP mutations in the 40K genome.

20K	Gene	Genome position	Ancestral nucleotide	Evolved nucleotide	Gene position	Codon change	Amino acid change
	<i>nrdD/treC</i>	4446077	T	G	−140/+254	–	–
	<i>treB</i>	4448669	G	T	789 (263)	CCC→CC <u>A</u>	Pro→Pro
	<i>pyrB</i>	4455062	T	G	611 (204)	G <u>A</u> T→G <u>C</u> T	Asp→Ala
	<i>valS</i>	4466019	A	C	852 (284)	TT <u>T</u> →TT <u>G</u>	Phe→Leu
	<i>valS</i>	4466781	T	G	90 (30)	GA <u>A</u> →GAC <u>C</u>	Glu→Asp
	<i>yjhC</i>	4487389	T	G	211 (71)	<u>T</u> AT→ <u>G</u> AT	Tyr→Asp
	<i>ECB_04147</i>	4490493	T	G	122 (41)	T <u>T</u> A→T <u>G</u> A	Leu→Stop
	<i>ECB_04147</i>	4490502	T	G	131 (44)	A <u>T</u> T→A <u>G</u> T	Ile→Ser
	<i>fecC</i>	4498025	T	G	417 (139)	GCA <u>A</u> →GCC <u>C</u>	Ala→Ala
	<i>sgcX</i>	4511435	A	C	355 (119)	<u>T</u> CC→ <u>G</u> CC	Ser→Ala
	<i>fimD</i>	4526771	A	C	762 (254)	AT <u>A</u> →AT <u>C</u>	Ile→Ile
	<i>fimH</i>	4530188	A	C	467 (156)	A <u>A</u> C→A <u>C</u> C	Asn→Thr
	<i>gntP</i>	4531921	A	C	290 (97)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>uxuB</i>	4534381	T	G	567 (189)	CG <u>T</u> →CG <u>G</u>	Arg→Arg
	<i>yjiK</i>	4544266	T	G	360 (120)	GA <u>A</u> →GAC <u>C</u>	Glu→Asp
	<i>yjiL</i>	4545297	T	G	307 (103)	<u>A</u> AA→ <u>C</u> AA	Lys→Gln
	<i>yjiN</i>	4547935	T	G	226 (76)	<u>A</u> TT→ <u>C</u> TT	Ile→Leu
	<i>yjiV†</i>	4552312	T	G	767	–	–
	<i>hsdM</i>	4560791	A	C	233 (78)	C <u>T</u> G→C <u>G</u> G	Leu→Arg
	<i>hsdR</i>	4562591	T	G	2146 (716)	<u>A</u> CC→ <u>C</u> CC	Thr→Pro
	<i>hpaB</i>	4570266	A	C	1474 (492)	<u>T</u> GC→ <u>G</u> GC	Cys→Gly
	<i>hpaD</i>	4577252	T	G	50 (17)	T <u>A</u> T→T <u>C</u> T	Tyr→Ser
	<i>yjiZ</i>	4583243	T	G	715 (239)	<u>A</u> AT→ <u>C</u> AT	Asn→His
	<i>yjiG</i>	4598130	A	C	251 (84)	G <u>A</u> A→G <u>C</u> A	Glu→Ala
	<i>osmY</i>	4601064	A	C	435 (145)	AA <u>A</u> →AA <u>C</u>	Lys→Asn
	<i>serB</i>	4613809	A	C	701 (234)	A <u>A</u> A→A <u>C</u> A	Lys→Thr
	<i>nadR</i>	4616396	G	A	868 (290)	<u>G</u> GC→ <u>A</u> GC	Gly→Ser
	<i>yjiY/lasT</i>	4628873	T	G	+171/−229	–	–

Supplementary Table 3. Details of SNP mutations in the 40K genome.

Black shading indicates that a mutation was also present in the 20K clone. Mutations in intergenic regions have the two flanking genes listed (e.g., *ybiN/potF*). Ancestral and evolved nucleotides refer to the top strand of the genome. For mutations in genes, the nucleotide positions of the reading frame are numbered in the direction of transcription, and numbers in parentheses are the corresponding amino-acid positions. For intergenic mutations, gene positions are nucleotides relative to each of the neighboring genes, where + indicates a distance relative to the stop codon of a gene translated toward the mutation and – indicates the position of the start codon of a neighboring gene that is oriented away from the mutation. †*fhiA*, *ycgH*, *hlyE*, *ydeU*, *yifN*, and *yjiV* are predicted to be pseudogenes. Mutations in these genes were not counted when tabulating synonymous and non-synonymous substitutions. ‡In addition to the *citC-gatZ* inversion, there appears to have been a second rearrangement involving the *ISI* element harboring the *insB*-15 mutation (genome position 2129116) at 40K. We were unable to PCR amplify this region to resolve whether it contained the same SNP mutation as the 20K clone.

Supplementary Table 4. Details of DIP mutations in the 40K genome.

20K	Gene or region	Mutation type	Genome start	Genome end	Size (bp)
	<i>mokC/nhaA::IS150</i>	IS-insertion§	16972	16974	3
	<i>mutT</i>	Insertion (C)	114034	114035	1
	<i>IS1::IS150</i>	IS-insertion§	241691*	241693*	3
	<i>ybaL</i> 18 (6)	Insertion (G)	475288	475289	1
	$\Delta(nmpC^{\dagger}$ -ECB_00513)	Deletion	547700	555923	8224
	<i>cusR</i>	Insertion (TTCGAC)	576331	576332	6
	<i>inv(citC-gatZ^{\dagger})</i>	Inversion \ddagger	634746	2128599	1493854
	<i>ldrA/ldrB::IS150</i>	IS-insertion§	1269593	¥	¥
	<i>gadB::IS1</i> (–)	IS-insertion§	1544689	1544697	9
	<i>pykF::IS150</i> (–)	IS-insertion§	1733647	1733649	3
	<i>ydiA</i> 243 (81)	Deletion	1764955	1764955	1
	<i>ynjI::IS150</i> (–)	IS-insertion§	1821525	1821527	3
	$\Delta(manB$ - <i>cpsG</i>)	Deletion	2031703	2054995	23293
	<i>ogrK</i> - ECB_02013	Deletion	2100308	2122453	22146
	$\Delta(gatZ$ - <i>yegX</i>)	Deletion	2129368	2137412	8045
	<i>menC::IS186</i> (–)	IS-insertion§	2322340	2322346	7
	<i>nupC/yfeA::IS186</i> (+)	IS-insertion§	2448493	2448498	6
	ECB_02621	Deletion	2792665	2792725	61
	<i>kpsD::IS150</i> (–)	IS-insertion§	3015771	3015774	4
	$\Delta gltB$	Deletion	3289962	3289977	16
	<i>glmU/atpC</i> –66/+286	Insertion (T)	3875632	3875633	1
	<i>kup/insJ</i> -5 +6/–49	Insertion (GG) ¶	3893551	3893552	2
	$\Delta(kup$ - <i>yieO</i>)	Deletion	3894998	3901931	6934
	$\Delta pfIC$ 342 (114)	Deletion	4126706	4126706	1
	<i>yjaH</i>	Deletion	4180211	4180211	1
	<i>fimA::IS186</i> (+)	IS-insertion§	4524522	4524527	6

Supplementary Table 4. Details of DIP mutations in the 40K genome.

Black shading indicates that a mutation was also present in the 20K genome. Gene names and positions are as in Supplementary Table 1. †*nmpC* and *gatZ* are pseudogenes interrupted by *IS1* elements. ‡For the *IS1*-mediated inversion between *citC* and *gatZ*, start and end positions are for the region bounded by the *IS* elements. There appears to be a second, unknown rearrangement involving these *IS1* elements in the 40K clone. Primers that amplify across the original and rearranged junctions in earlier clones consistently fail to produce any PCR product from 40k genomic DNA. §For *IS*-element insertions, start and end positions are for the duplicated target sequence, and the resulting size corresponds to the number of target nucleotides, not the *IS* element. The orientation of the new *IS* relative to the genome's top strand is shown as + or – in the gene column. *The *IS150* insertion into an *IS1* element could have occurred in the same sequence context in any of eight different *IS1* copies. The start and end coordinates shown are for a representative example. ¶The 20K genome has an insertion of a single G at the same site in the *kup/insJ-5* intergenic region. ¥We were unable to resolve both boundaries of this putative *IS*-element insertion from WGS data or with targeted PCR amplifications. This region of the genome contains repeated DNA sequences, and it is possible that the newly detected *IS150* junction is part of a more complicated duplication or rearrangement event.