

Legends to supplementary figures

Figure S1. Confirmation of duplication in M184 and three R6^{M184} transformants by amplification of the duplication junction. **A.** Location of PCR primers in R6 sequence. Primers designed for the predicted duplication junction (solid arrows) bind divergently in R6 so will only produce an amplicon if a genomic rearrangement moves the primer binding sites such that the primers amplify convergently. As a control for DNA template integrity, the intergenic region between *hexA* and *patA* was amplified simultaneously (*hexA* primer indicated by dashed line). **B.** Results of PCR experiments described above. Upper panel: junction PCR. Lower panel: control PCR. Lane 1, R6; Lane 2, M184; Lane 3, T1; Lane 4, T2; Lane 6, T3.

Figure S2. Confirmation of duplication in M184 and three R6^{M184} transformants by production of long and short PCR amplicons. **A.** Location of *patA* and *hexA* primers in predicted M184 sequence. **B.** Amplification with a long extension time results in long and short PCR amplicons from M184 (lane 3) and transformants T1, T2 and T3 (lanes 4, 5 and 6) but not from R6 (lane 2). Lanes 1 and 8 contain Hyperladder 1kb (Bioline)

Figure S3. Alignment of 50 bp of sequence surrounding the start, end and junction point of the duplicated region in M184. Conserved bases are indicated by dark shading. Boxed residues in the sequence surrounding the end point of the duplication form an inverted repeat.

Figure S1A

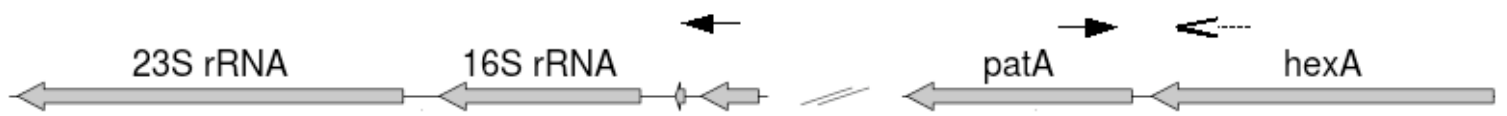


Figure S1B

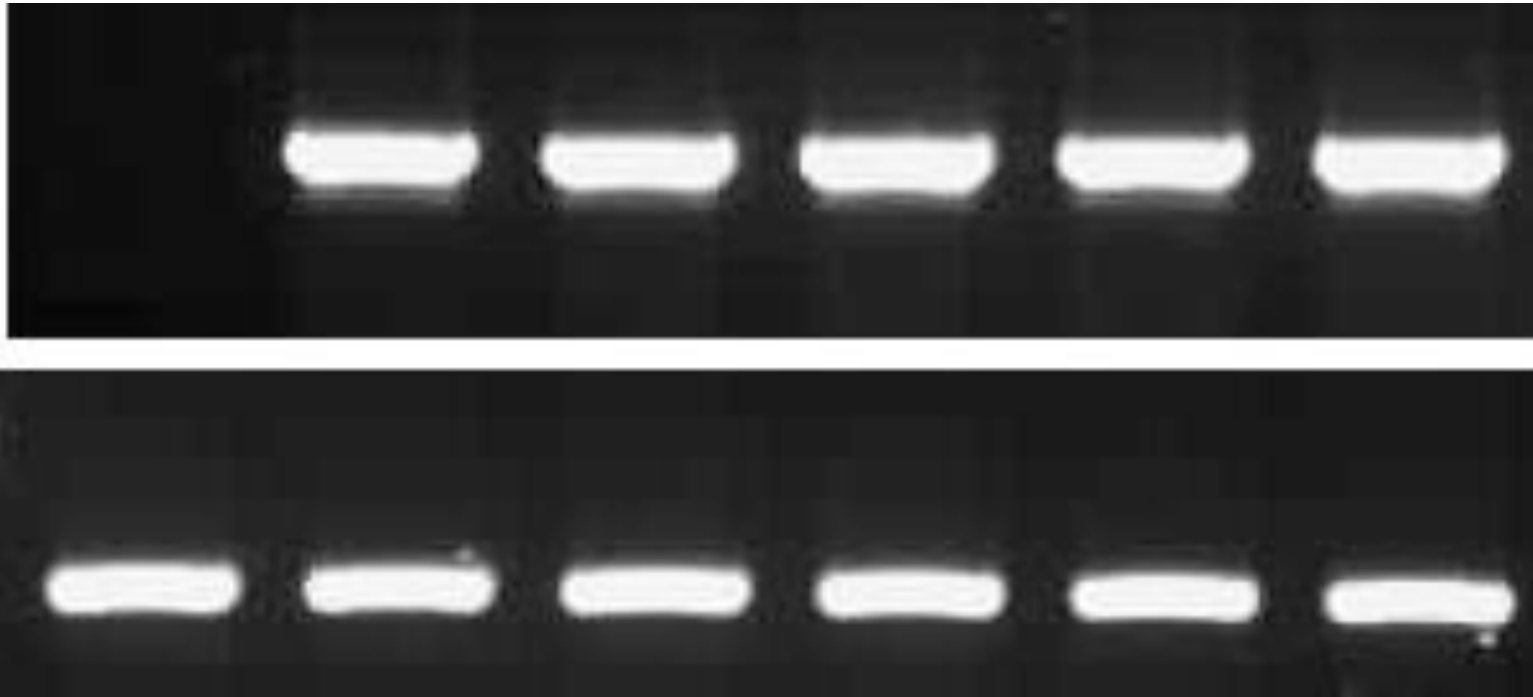


Figure S2A

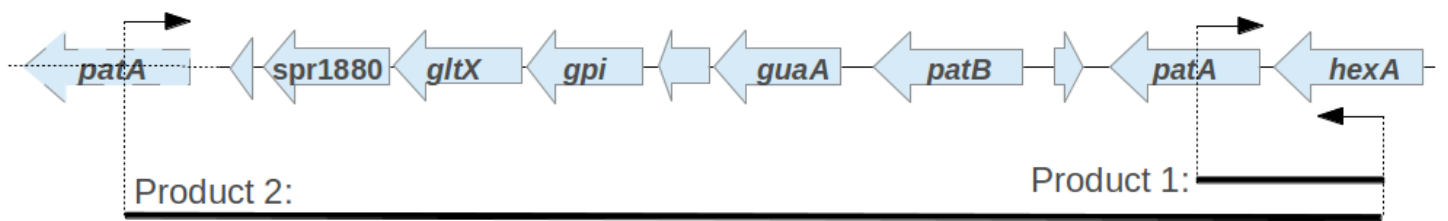


Figure S2B

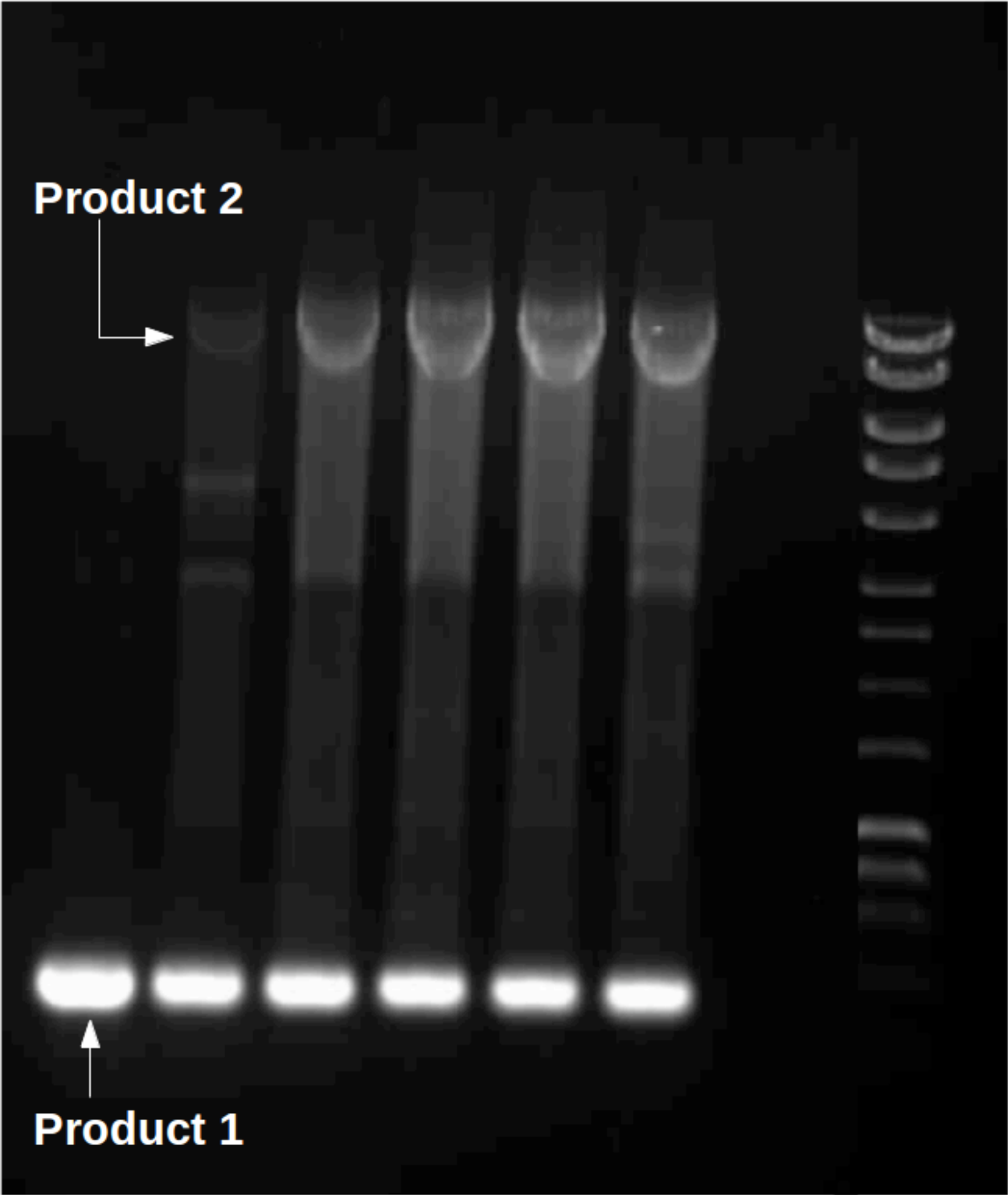


Figure S3

duplication_start
duplication_junction
duplication_end

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1 GTATAAATATGACACCTATGCAGGTTATGAATGTCCTAGTAGAGTTAAAAC  
1 ACCAATGTGCAGGGCGCTACAACATATGAATGTCCTAGTAGAGTTAAAAC  
1 ACCAATGTGCAGGGCGCTACAACATAAGTTGTAGTACTGAACAATGAAAA
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Table S1. Details of mutations found in M184, M500, M501 and M503 relative to R6

Strain	Position	Base change	Mutation type ¹	Gene	Description	Protein change ²
M184	269365	C-T	non-syn	sulB	dihydrofolate synthetase	Ser133Leu
	263291	-8	del	-	-	-
	387172	C-A	int	-	-	-
	460764	G-T	trunc	hrcA	heat-inducible transcription repressor	Glu269*
	569420	G-R	int	-	-	-
	634993	G-T	non-syn	lctO	lactate oxidase	Gly10Val
	635851	G-T	non-syn	lctO	lactate oxidase	Gly296Val
	808808	T-C	non-syn	spr0807	hypothetical protein	Ile55Thr
	974512	+2	ins	murZ	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	trunc
	1142521	G-T	non-syn	topA	DNA topoisomerase I	Ala431Asp
	1167599	G-A	non-syn	pyrP	Uracil permease	Val124Ile
	1327871	G-T	non-syn	tufA	elongation factor Tu	Thr97Asn
	1563763	C-A	non-syn	spr1582	hypothetical protein	Ala35Ser
	1570591	+1	ins	spr1591	hypothetical protein	-
	1595105	G-T	trunc	spr1620	ABC transporter SBP - sugar transport	Tyr68*
	1670128	G-A	non-syn	treP	trehalose-specific PTS	Thr28Ile

	1845480	G-A	non-syn	spr1875	hypothetical protein	Ala63Val
	1857615-8446	10 SNPs	non-syn	gltX	glutamyl-tRNA synthetase	various
M500	141189	C-A	int	-	-	-
	541249	A-G	syn	murM	Ser/Ala adding enzyme, peptidoglycan biosynthesis	-
	792564	A-T	int	-	-	-
	1052448	C-A	syn	spr1057	hypothetical protein	-
	1282189	C-T	int	-	-	-
	1826248	G-T	non-syn	spr1850	hypothetical protein	Gln61Lys
M501	599258	C-A	non-syn	spr0583	hypothetical protein	Ala78Glu
	635793	G-T	non-syn	lctO	Lactate oxidase	Val277Leu
	969735	G-T	non-syn	obgE	GTPase implicated in regulation of chromosomal replication	Ala12Ser
M503	391425	G-A	syn	gatA	aspartyl/glutamyl-tRNA amidotransferase subunit A	-
	599258	C-A	non-syn	spr0583	hypothetical protein	Ala78Glu
	635793	G-T	non-syn	lctO	Lactate oxidase	Val277Leu
	969735	G-T	non-syn	obgE	GTPase implicated in regulation of chromosomal replication	Ala12Ser
	1012631	C-T	non-syn	glgC	glucose-1-phosphate adenylyltransferase	Thr149Ile
	1426032	C-T	syn	oxlT	oxalate:formate antiporter	-
	1474250	C-G	non-syn	-	hypothetical protein	Gly284Arg

1550514	C-T	non-syn	scrR	sucrose operon repressor	Arg106Cys
1643773	G-A	non-syn	adhB	zinc-containing alcohol dehydrogenase	Pro84Leu

¹ non-syn, non-synonymous; syn, synonymous; trunc, truncation; int, intergenic; ins, insertion; del, deletion. ² *, stop codon.