## *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 amplimer 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 amplimers. **A.** Location of *patA* and *hexA* primers in predicted M184 sequence. **B.** Amplification with a long extension time results in long and short PCR amplimers 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

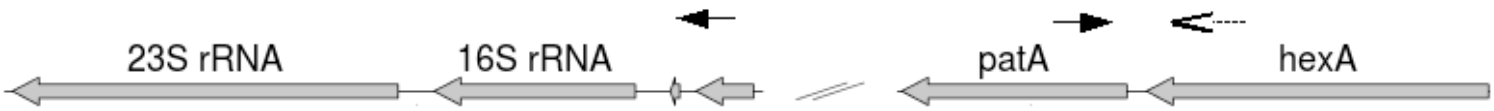

Figure S1B

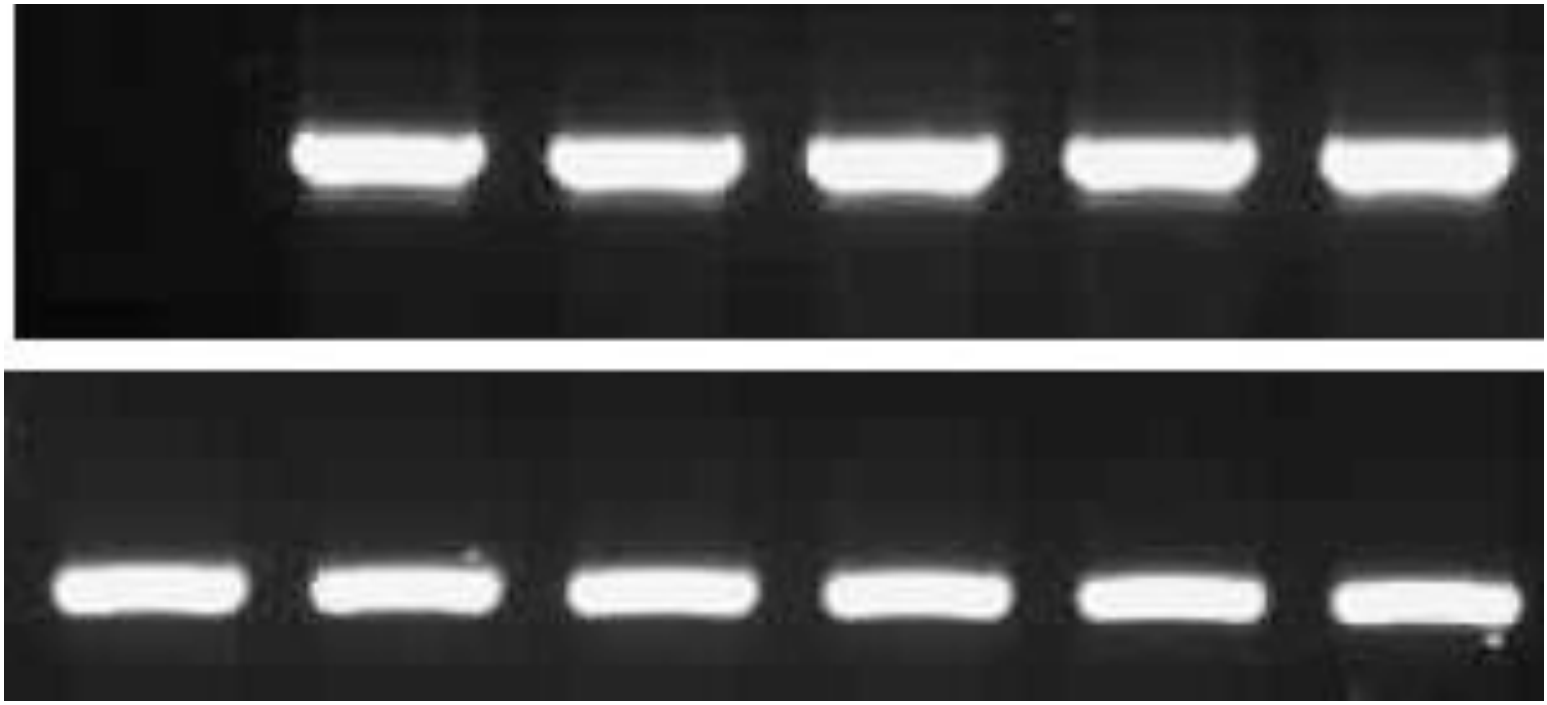

Figure S2A

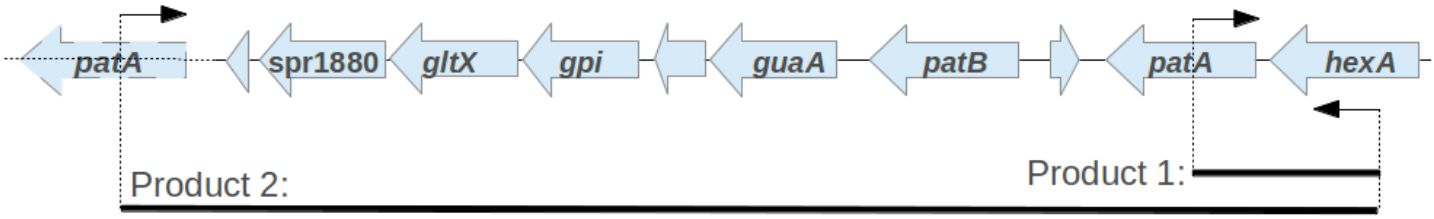
patA
spr1880
gltX
gpi
guaA
patB
patA
hexA
Product 2:
Product 1:

Figure S2B

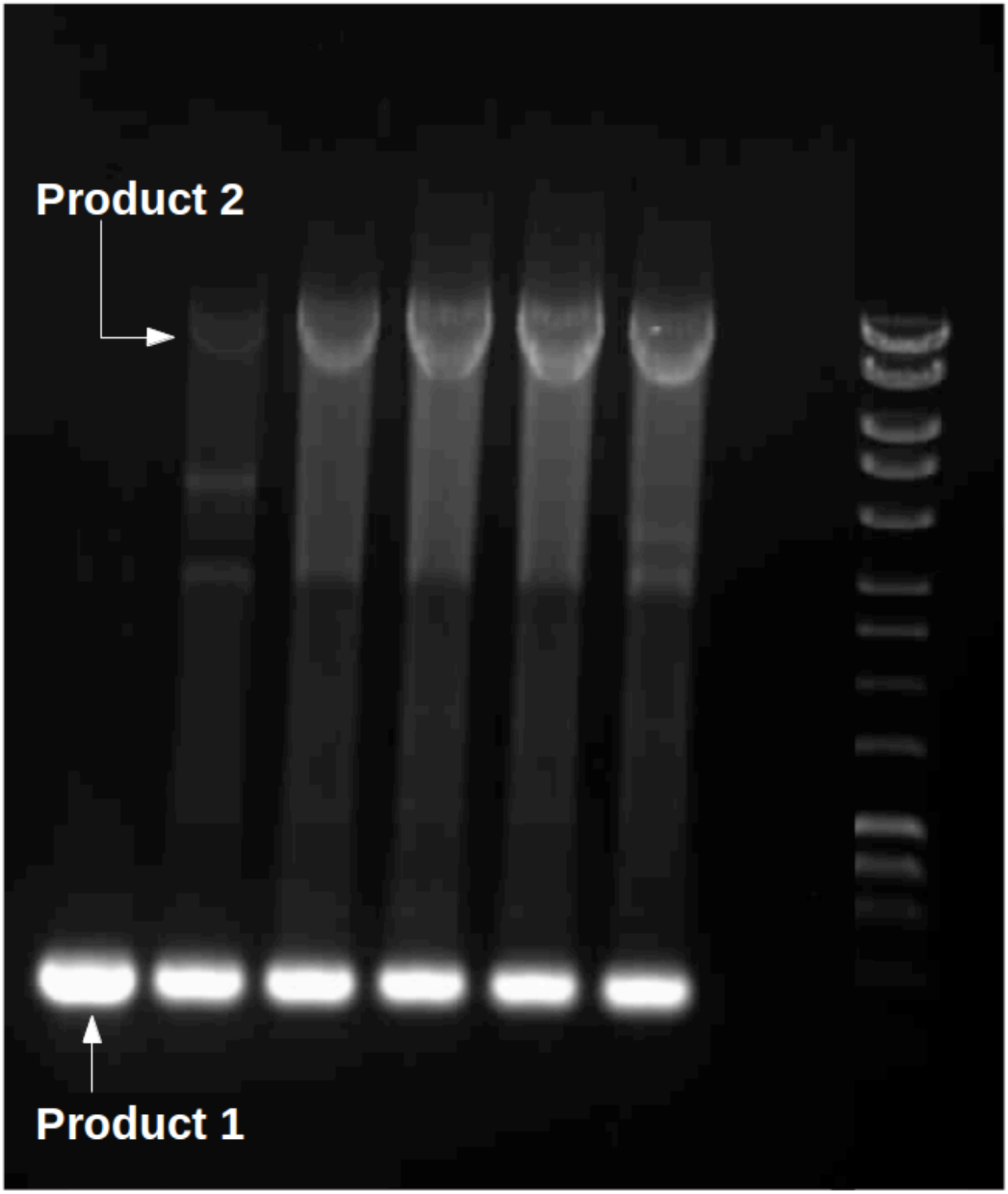

Figure S3

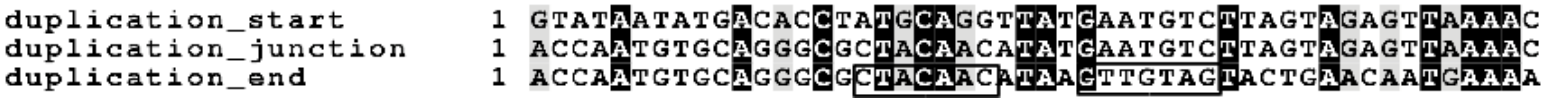
duplication_start 1 GTATAATATGACACCTATGCAGGTTATGAATGTCTTAGTAGAGTTAAAAC
duplication_junction 1 ACCAATGTGCAGGGCGCTACAACATATGAATGTCTTAGTAGAGTTAAAAC
duplication_end 1 ACCAATGTGCAGGGCGCTACAACATAAGTTGTAGTACTGAACAATGAAAA

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.