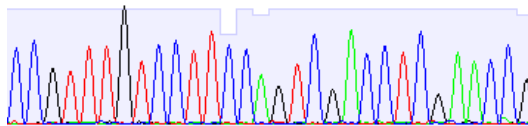


Region 1

EXPECTED: **CGGTTGAGGTTCGACTGGAAGGACAAACGG**

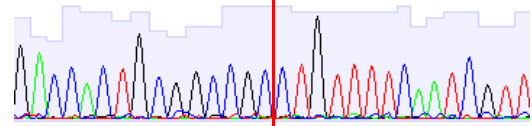
VERIFIED: CCGTTTGTCCCTCCAGTCGACCTCGAACCG
(reverse-complement sequence)



Region 3

EXPECTED: **GACCACTGCGGCCGCTGTTTTCAATCGTT**

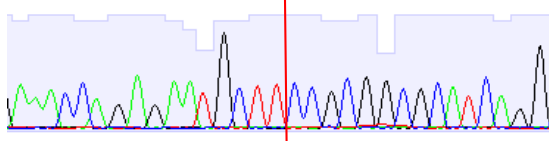
VERIFIED: GACCACTGCGGCCGCTGTTTTCAATCGTT



Region 2

EXPECTED: **AAACCAGAGAATGCTTCCGCGGCATCAGG**

VERIFIED: AAACCA GAG AAT GCT TCC GCG GCG CAT CAG G



Region 4

EXPECTED: **TTTGGCAGTCAGAAACTAGAAAGCTTTTTTG**

VERIFIED: TTTGGCAGTCAGAAACTAGAAAGCTTTTTTG

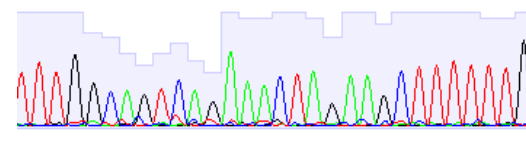


Figure S1 Schematic of *mec-8* locus deletion strain generation and validation by PCR and sequencing. Upper panels display genomic *mec-8* locus (in black), repair template with 5' and 3' homology arms flanking loxP sites and dual marker cassette (homology arms labeled as red lines), and the resulting recombined locus where most of the *mec-8* locus is replaced with the dual marker cassette. PCR primers (red and black arrows) were designed to selectively amplify recombinant-specific products on the 5' and 3' sides of the breakpoints. These PCR products (displayed below the arrows as red and black lines) then underwent Sanger sequencing to confirm expected editing. The boxed regions highlight relevant boundaries between vector-encoded homology arms and genomic regions outside of homology arms (Regions 1 and 4), or boundaries between dual marker cassette and deletion sites (Regions 2 and 3). Sequencing traces below demonstrate expected base calls if editing and repair occurred precisely as planned. Red vertical lines in Region 2 and 3 traces indicate deletion breakpoints.