Supplementary material

Fig. S1. A genetic system for gene disruption in Msm. Plasmid pML2424 is designed to facilitate the isolation of mutants that result from homologous recombination. It confers hygromycin resistance, has a temperature-sensitive mycobacterial origin of replication and a ColE1 origin used for replication in *Escherichia coli*. In addition, it expresses both GFP and RFP from constitutive mycobacterial promoters. Consequently, at a restrictive temperature (42°C), double cross-over clones that result from homologous recombination express GFP, but not RFP, and can be easily detected on plates using fluorescence microscopy or a fluorescence imager. To facilitate the isolation of such mutants, pML2424 carries the *sacB* gene, enabling counter selection on plates containing sucrose (10 % W/V). In our study, the upstream (US) flanking region was amplified by PCR and cloned into the *Spe*I and *Swa*I cut sites of plasmid pML2424. Similarly, the downstream (DS) flanking region was cloned into the *Pac*I and *Nsi*I cut sites. Following successful deletion using pML2424, plasmid pML2714 can be used to facilitate excision of the *gfp-hyg* cassette from the chromosome via *loxP* site-specific recombination.

Fig. S2. Knocking out the *ect* operon. (A) Schematic diagram of the homologous recombination event that led to replacement of the chromosomal copy of the *ect* operon with the *gfp-hyg* cassette of pML2424. Using plasmid pML2714, a *loxP* site-specific recombination catalyzed by the Cre recombinase results in excision of the *gfp-hyg* cassette from the Msm chromosome. (B) PCR analyses of the wild-type and the Δ*ect* mutant strains. In the right panel, the PCR reactions were with internal *ect* primers [dotted lines; primers 11 and 12 (Table S1)]. In the left panel, the PCR reactions were with external *ect* primers [solid lines; primers 13 and 14 (Table S1)]. (C) Southern analysis was carried out on *Bam*HI-digested chromosomal DNA preps from the wild-type and the Δ*ect* mutant strains. The external probe used in the assay [right panel; primers 15 and 16 (Table S1)] hybridized with a DNA fragment comprising the upstream flanking region. The internal probe [left panel; primers 17 and 18 (Table 1)] hybridized with a DNA fragment from within the *ect* operon.

Fig. S3. Complementation of the *ect* operon deletion. The Msm *ect* operon including 875 bp upstream the *ectA* translation start site was cloned into the *Kpn*I – *Xba*I cut-sites of the mycobacterial shuttle vector, pMV206. The cloned plasmid was transformed into the Δ*ect* mutant and a spot-test assay was carried out (as in Fig. 3A) on plates containing 1 M NaCl and kanamaycin (10 μg/mL). As controls, mutant and wild-type strains were used, both carrying empty pMV206 vectors.

Table S1. Primers used for analysis in this study

#	Primer name	Primer sequences (5'→3')	Hybridized DNA
1	ectB F	TCATGCCGTGAAATGCGT	*ectB*
2	ectB R	GGTGGAATCCGCGCTG	*ectB*
3	sigA F	GAAGACACCGACCTGGAACT	*sigA*
4	sigA R	GACTCTTCCTCGTCCCACAC	*sigA*
5	ectAB F	ACCGATCGGGGTCTGAGG	*ectAB* junction
6	ectAB R	AACCTCAGGCAGGTCGGA	*ectAB* junction
7	ectBC F	CGATCGCAGTCACCTTGA	*ectBC* junction
8	ectBC R	AAACCCACCTTGTCGTCG	*ectBC* junction
9	ectCD F	CATGCTGTGCGTCTTCAA	*ectCD* junction
10	ectCD R	GGTGTCGTCTGGGTGGTC	*ectCD* junction
11	ectC F	AGGAGAACACCCATGATT	*ectC*
12	ectD R	GAAATCCGAGTGCCAATA	*ectD*
13	ect US F2	AGTACCTGTAGGACCCGC	*ectA* up-stream region
14	ect DS R2	TTGATGATGCCGCGTTTG	*ectD* down-stream region
15	ect US F	ATGACTATTTAAATTCCAAGTTCTC GATGAAC	*ectA* up-stream region
16	ectUS R2	TTTTCGACAGCGTCAGA	*ectA* up-stream region
17	ectA int F	CATCACTGGTTACCACCC	*ectA*
18	ectB int R	AGTAGCTACGGACCTCGG	*ectB*

Fig. S1

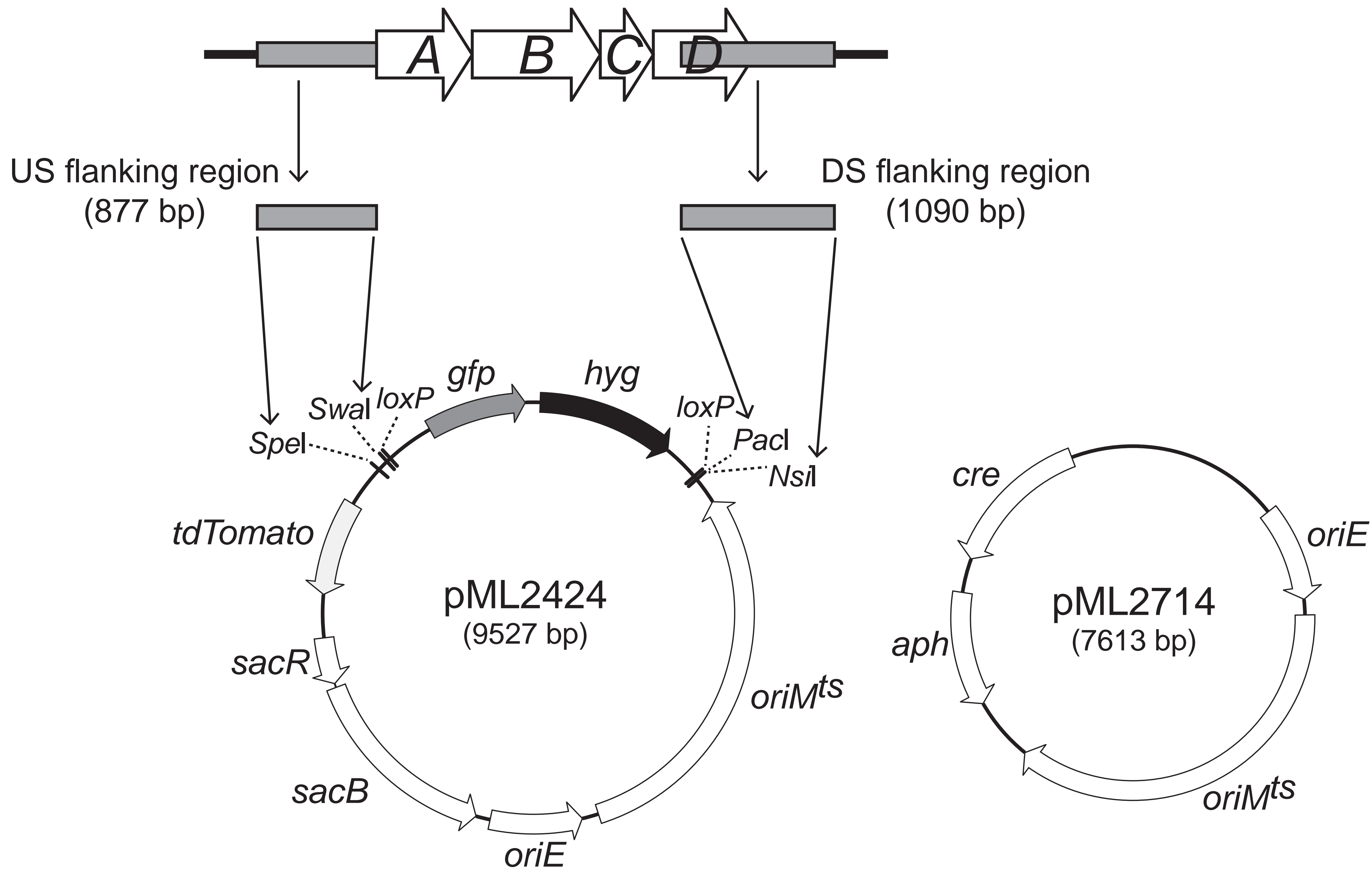
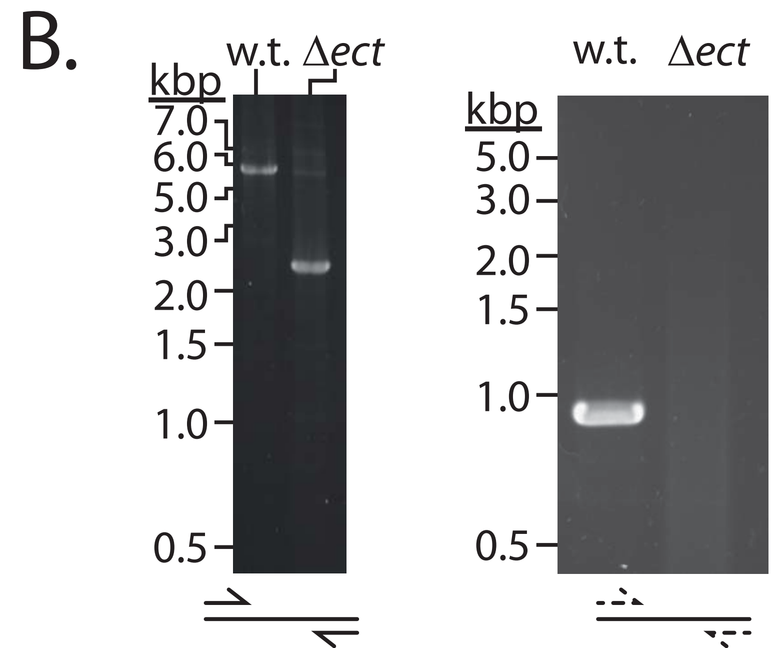


Fig. S2



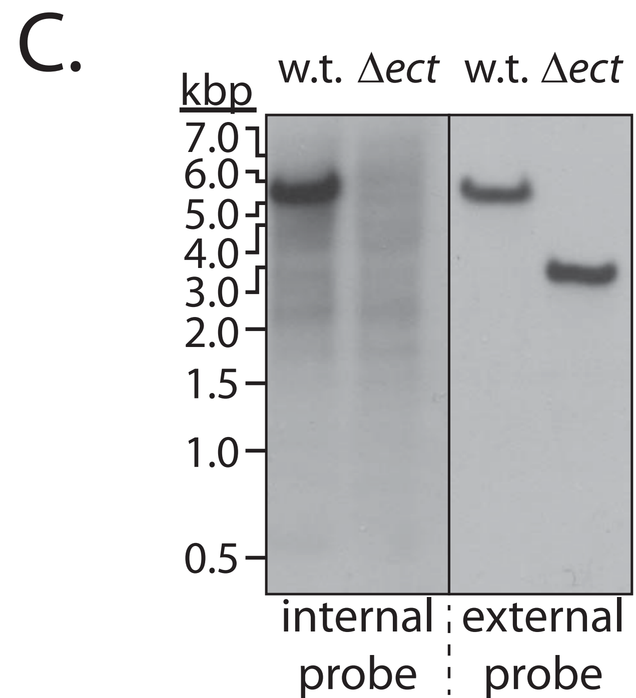


Fig. S3