molecular microbiology

Supplementary Information

CroS$_{R391}$, an ortholog of the λ Cro repressor, plays a major role in suppressing polV$_{R391}$-dependent mutagenesis

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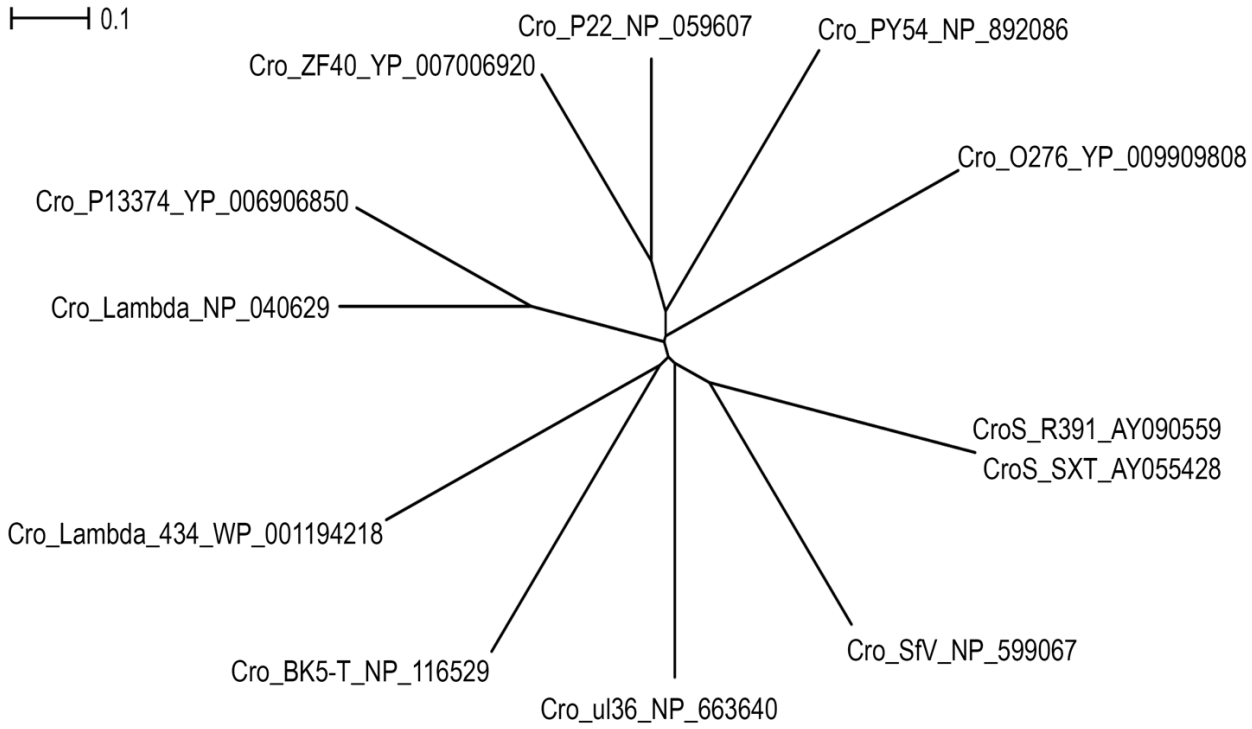

Figure S1. A multiple sequence alignment of the CroS protein from ICE391 and various other homologous proteins was performed using the ClustalW algorithm in MacVector (version 15.5.4). The alignment was exported from MacVector as a Phylip file. This Phylip file was imported into the SplitsTree4 (version 4.14.4) and an unrooted phylogenetic tree was generated by setting the distance method to BioNJ. This unrooted phylogenetic tree indicates the evolutionary relatedness of the CroS transcriptional repressor from R391 with other various related Cro-like transcriptional repressors. As can be seen, $CroS_{R391}$ is 100% identical to $CroS_{SXT}$. Protein sequences were obtained from the following Genbank sequence files: AY090559 (R391 nucleotides 87195-87443 CroS); AY055428 (SXT s086 CroS); NP_599067 (phage SfV); NP_663640 (phage ul36); NP_116529 (phage BK5-T); WP_001194218 (λ P434); NP_040629 (λ phage); YP_006906850 (phage P13374); YP_007006920 (phage ZF40); NP_059607 (P22); NP_892086 (phage PY54); YP_009909808 (phage O276).

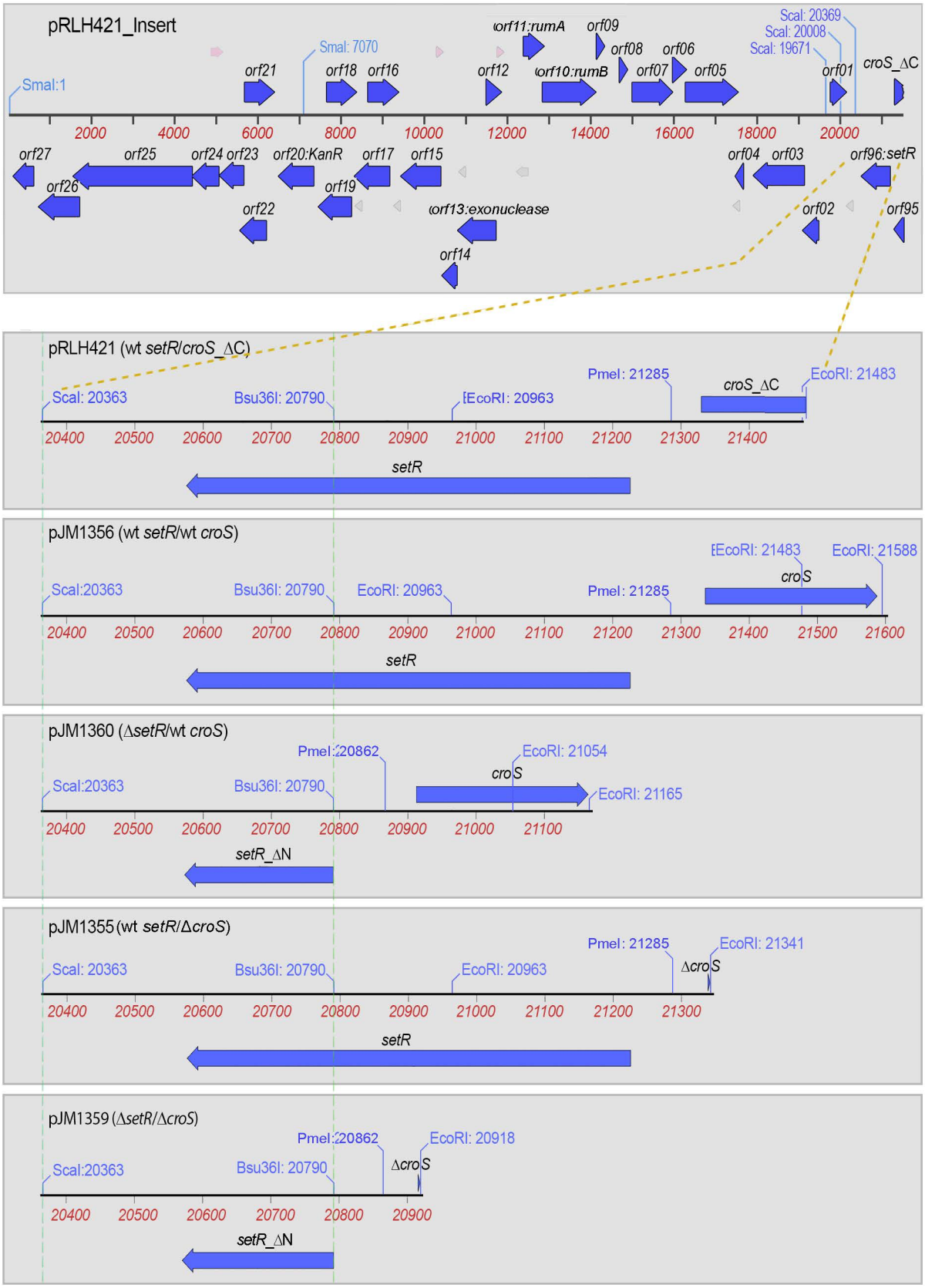

Figure S2: Cartoon of the DNA inserts in pRLH421 and its *setR* and *croS* derivatives.

The *rumAB* operon was originally subcloned in 1993 into the low-copy vector, pGB2, as a partial ~21.5 Kb *Eco*RI digest of episomal R391. Plasmids pJM1356 (*setR*$^+$ *croS*$^+$), pJM1360 (Δ*setR croS*$^+$), pJM1355 (*setR*$^+$ Δ*croS*), and pJM1359 (Δ*setR* Δ*croS*) were generated in pRLH421 and all contain 20.3 Kb of R391 encompassing orfs 1-27 (top panel). Smaller plasmid derivatives expressing just *setR,* or *croS*, were generated by deleting the 20.3 KB R391 DNA between the *Sma*I site (at position #1) and the *Sca*I site (at position #20363), to generate pJM1366 (*setR*$^+$ *croS*$^+$), pJM1368 (Δ*setR croS*$^+$), pJM1365 (*setR*$^+$ Δ*croS*), and pJM1367 (Δ*setR* Δ*croS*), respectively.

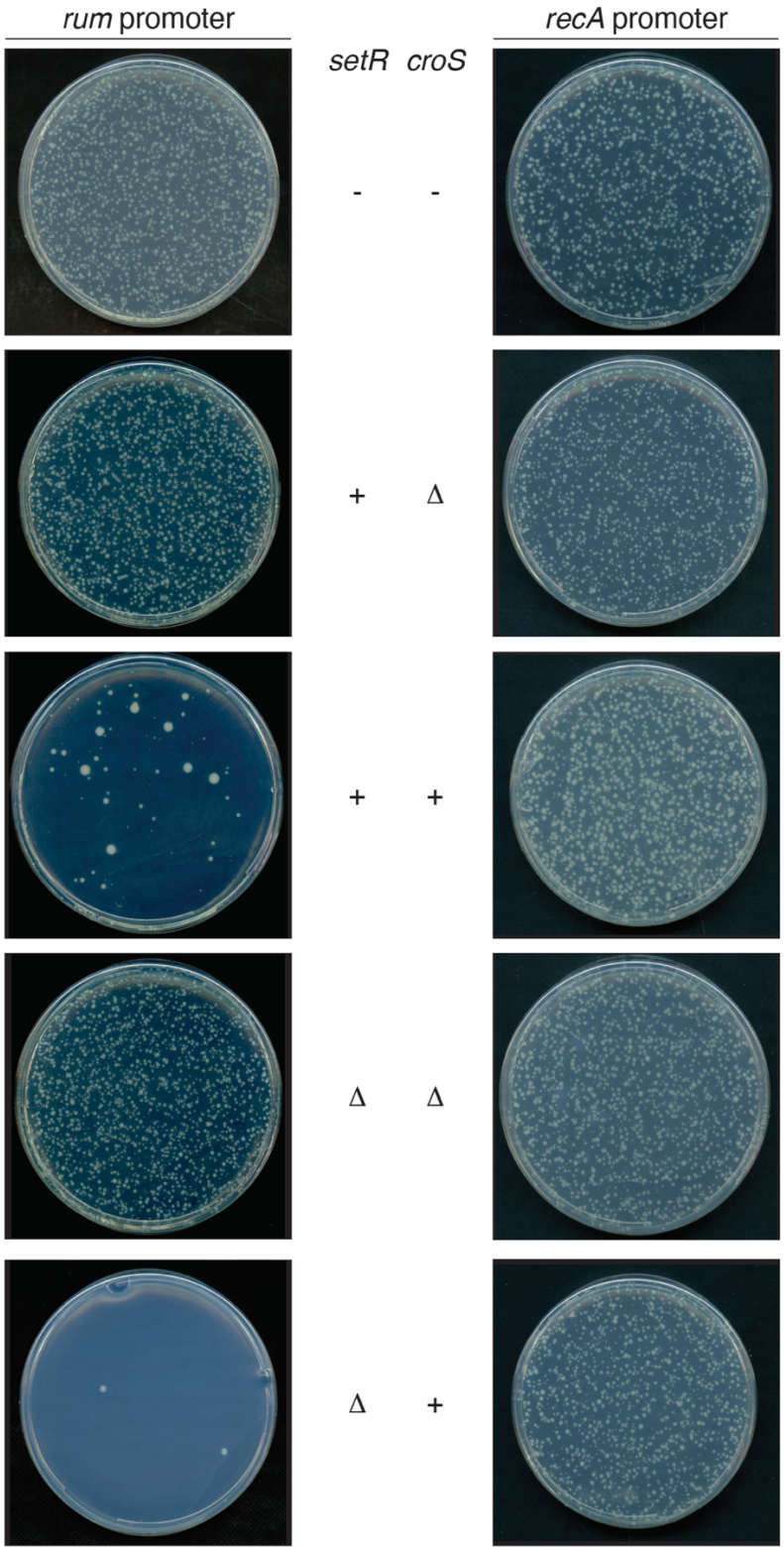

Figure S3. Visualization of *rumAB*-dependent spontaneous mutagenesis when expressed from their native promoter (pJM1378), or from the *recA* promoter (pJM1467) and in the presence of compatible *setR*/*croS* plasmids.

pJM1365 (*setR*$^{+}$ Δ*croS*), pJM1366 (*setR*$^{+}$ *croS*$^{+}$), pJM1367 (Δ*setR* Δ*croS*) or pJM1368 (Δ*setR croS*$^{+}$). Images are representative of the data presented in the form of a histogram in Figures 4A and 5A.

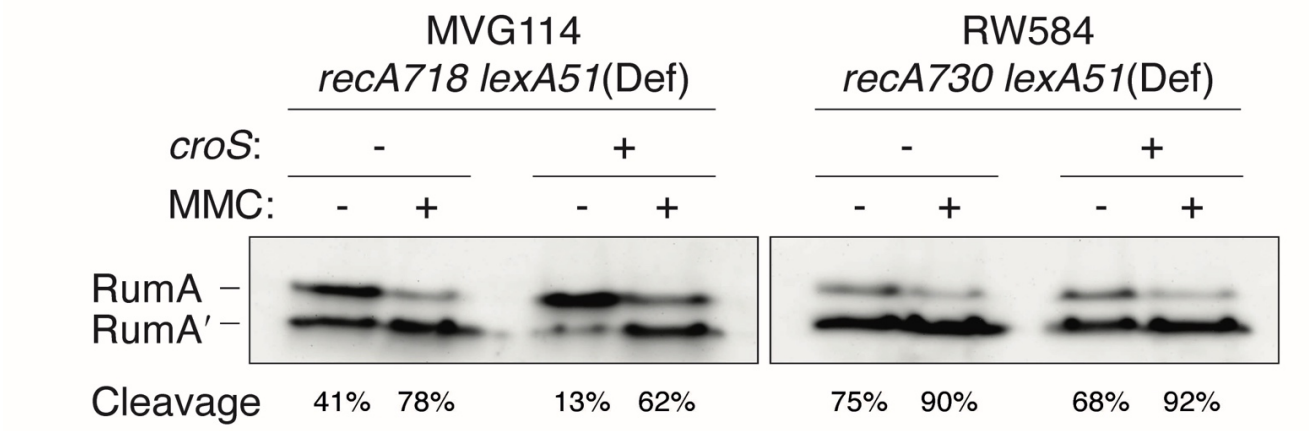

Figure S4: Western blot showing cleavage of RumA in an undamaged, or damaged (Mitomycin C-treated) *recA718 lexA*(Def) or *recA730 lexA*(Def) strains in the absence, or presence, of the CroS expressing plasmid, pJM1368).

Least cleavage is observed in the undamaged *recA718 lexA*(Def) strain MVG114, but significantly higher levels of cleavage are observed in the same strain exposed to the DNA damaging agent, Mitomycin C. Cleavage of RumA is much more efficient in both the undamaged and damaged *recA730 lexA*(Def) strain, RW584, and no inhibition of cleavage is observed in the presence of CroS in this strain background.

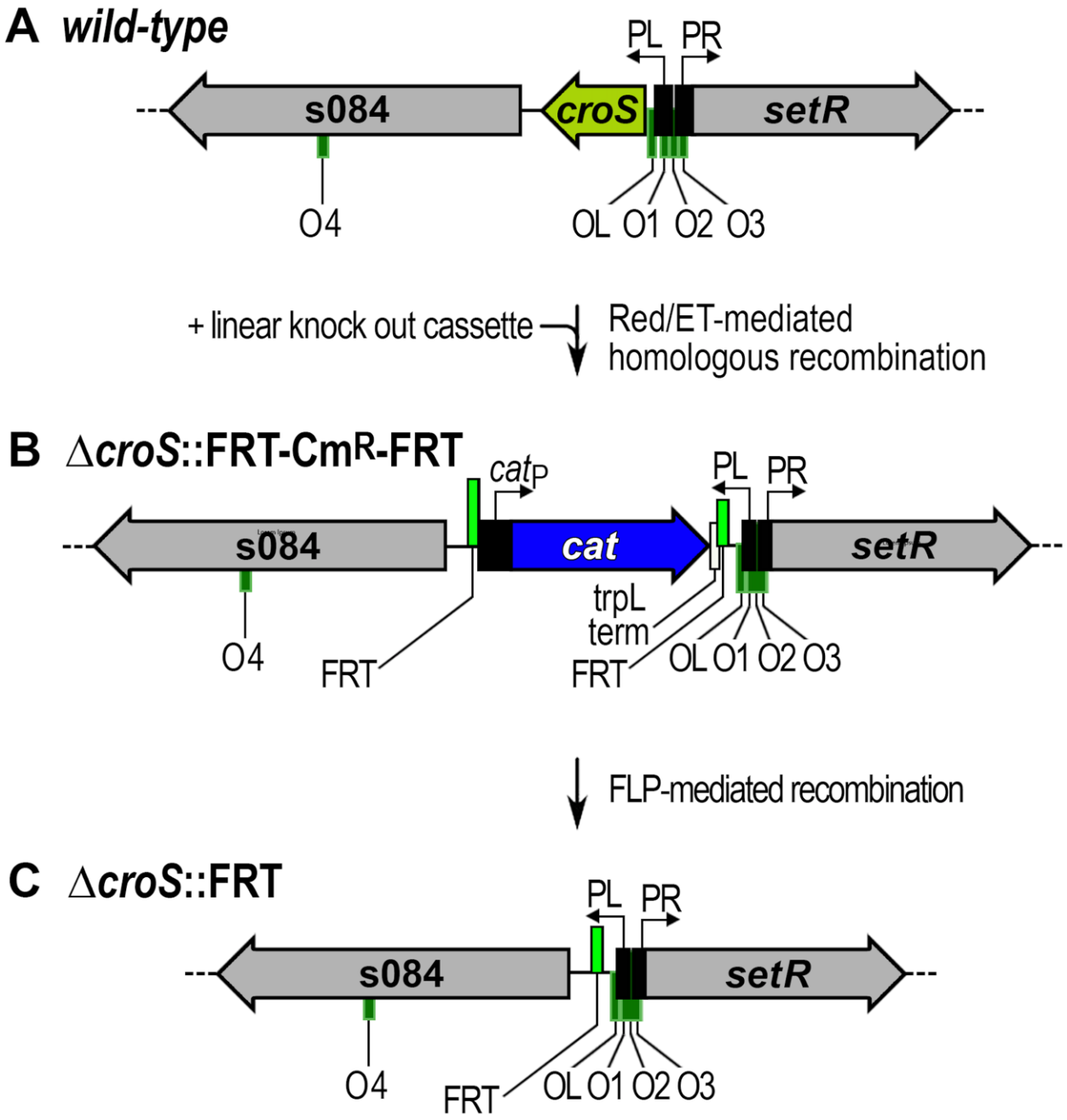

Figure S5: Workflow for the construction of a Δ*croS* allele.
A. In the first recombination step, a linear *knock out* cassette was used to replace the wild-type *croS* gene via Red/ET recombination. **B**. This resulted in a chloramphenicol-resistant intermediate strain with the genotype Δ*croS*::FRT-Cm^R-FRT. **C**. In a second step, the selection marker was removed in a FLP recombinase-mediated fashion, leaving a single FRT site at the former *croS* locus.