Supporting Information for:

Stringent Response Processes Suppress DNA Damage Sensitivity Caused by Deficiency in Full-Length Translation Initiation Factor 2 or PriA Helicase

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SI Figures

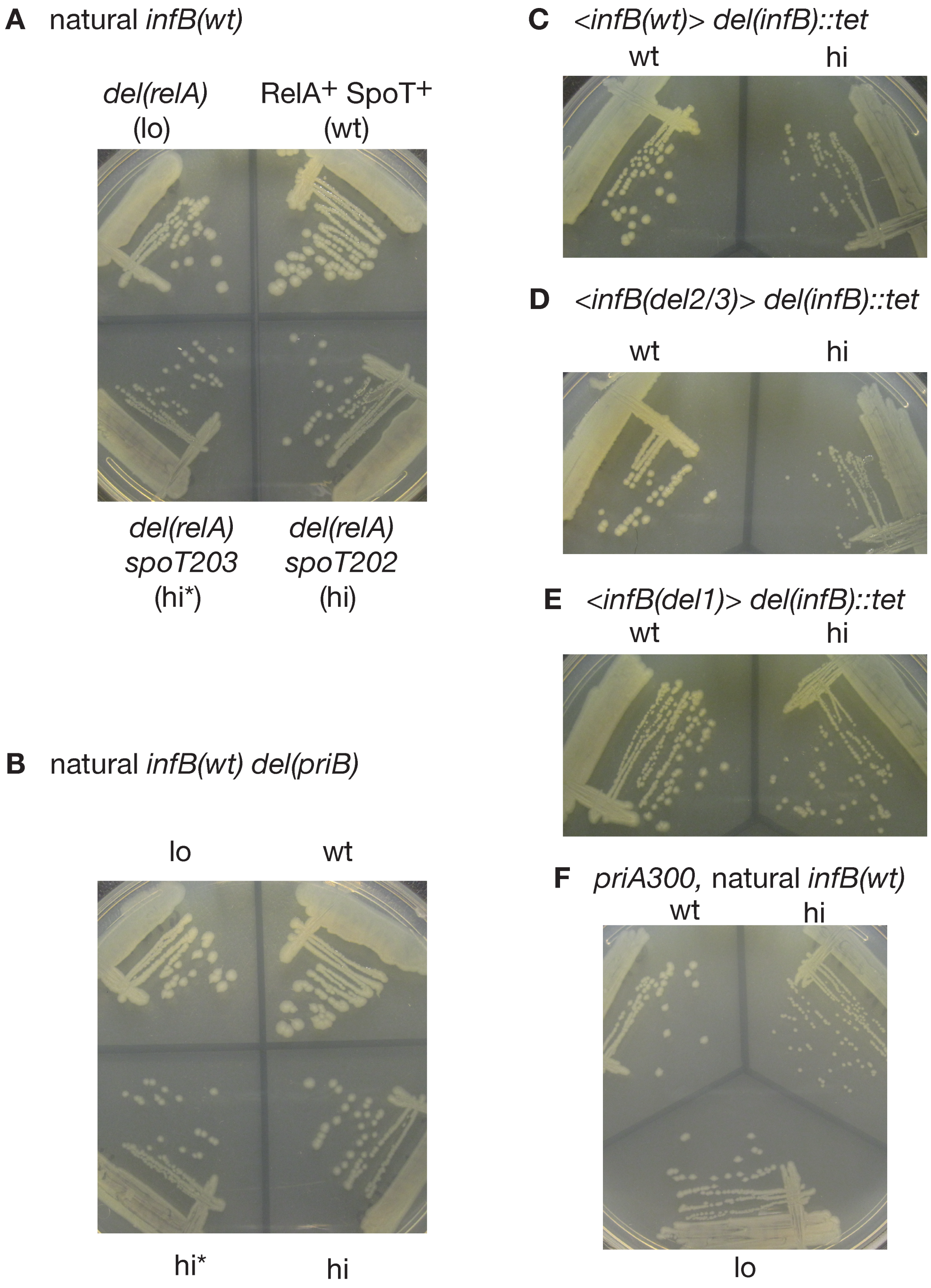

Figure S1. **Effect of the various alleles affecting ppGpp levels on colony size.**
A. Effect of the lo to hi* ppGpp background for a strain with wild-type *infB* and restart functions. Strains were streaked out on LB plates and incubated at 37°C for 20-24 h. The specific strains are (clockwise from lo, upper left): GTN701, GTN932, GTN722, GTN723.

B. Effect on a *del(priB)* strain. The strains are (clockwise from lo, upper left): GTN700, GTN394, GTN724, GTN726.
C. The <*infB(wt)*> strain at wt and hi ppGpp. The strains are GTN1050 and GTN1703, respectively.
D. The <*infB(del2/3)*> strain at wt and hi ppGpp. Strains: GTN1115 and GTN1705, respectively.
E. The <*infB(del1)*> strain at wt and hi ppGpp. Strains: GTN1114 and GTN1704, respectively.
F. The *priA300* strain at lo to hi ppGpp. Strains: GTN1713, GTN381, and GTN1717, respectively.

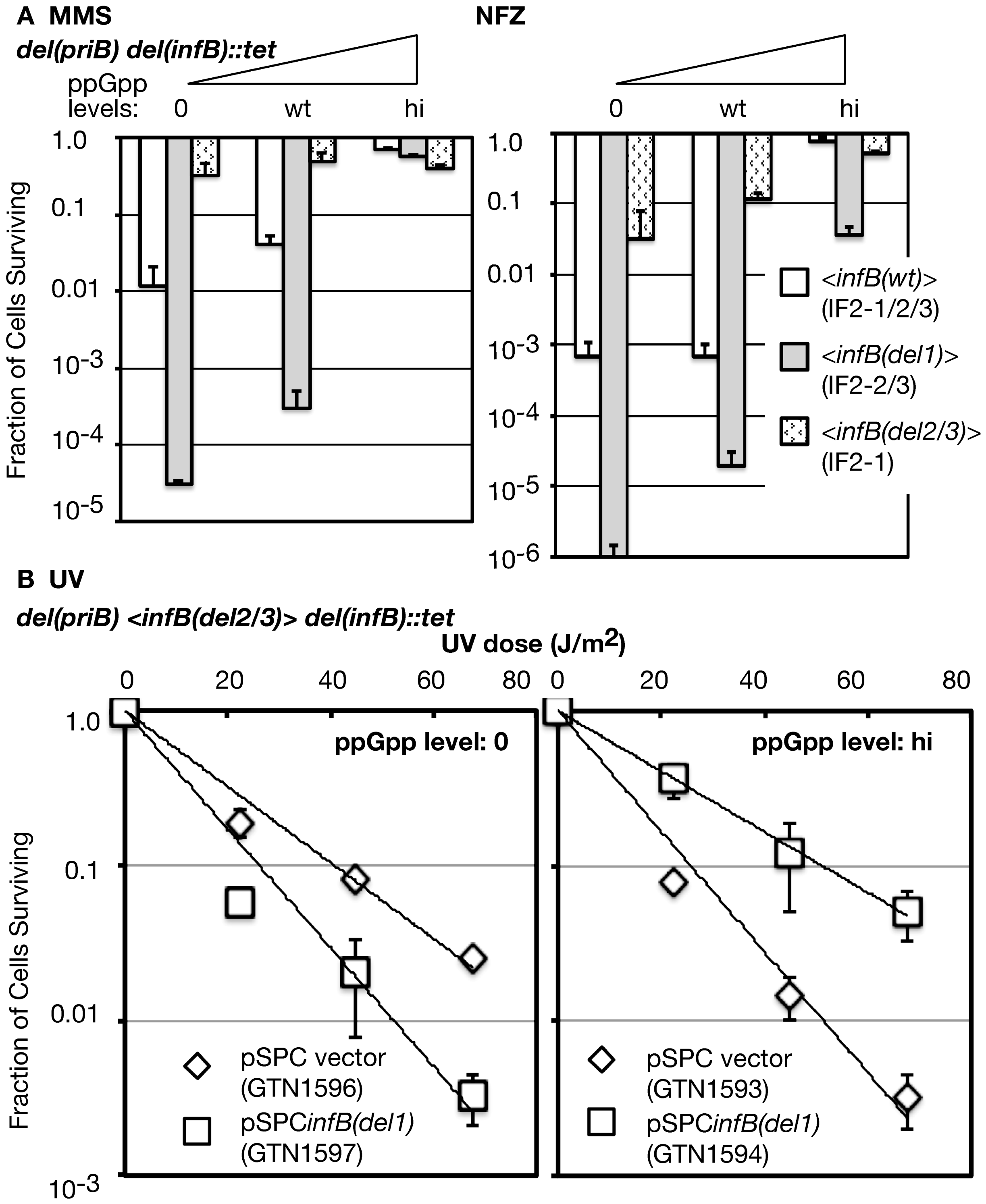

Figure S2. **The effect of ppGpp on DNA-damage sensitivity of *del(priB)* strains expressing IF2-1 and IF2-2/3.**

A. MMS and NFZ sensitivity. Survival of the various <*infB*> *del(priB)* strains with the indicated ppGpp background is shown. In the legend the IF2 isoforms expressed by each <*infB*>

allele are indicated in parenthesis. The specific strains, listed in ascending ppGpp order for each group, are *<infB(wt)> del(priB)*: GTN1498, GTN1122, GTN1502; *<infB(del1)> del(priB)*: GTN1537, GTN1117, GTN1503; *<infB(del2/3)> del(priB)*: GTN1499, GTN1119, GTN1505. B. UV sensitivity. Complementation of the *<infB(del2/3)* strain with pSPC*infB(del1).* The pSPC*infB(del1)* expresses IF2-2/3 while the single copy *<infB(del2/3)* allele expresses IF2-1. The pSPC vector (diamonds) refers to the empty plasmid vector, the strain having only the single-copy *<infB(del2/3)* allele.

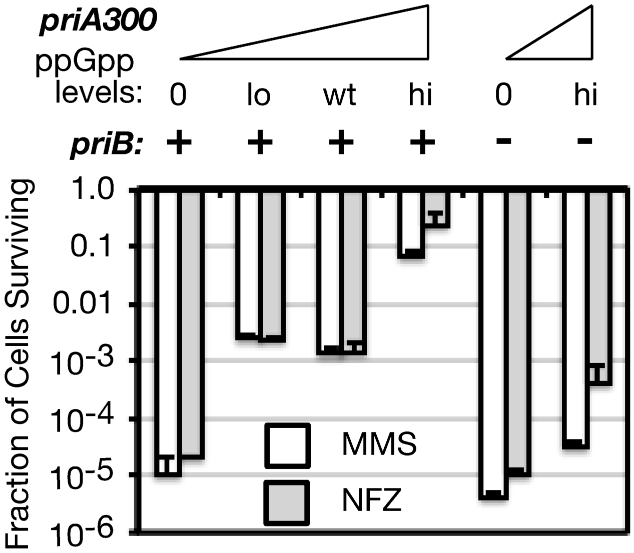

Figure S3. **MMS and NFZ sensitivity of the *priA300* mutant at various ppGpp levels.** The strains have the indicated ppGpp background and are PriB$^+$ (+) or *del(priB)* (-). The results of examining MMS sensitivity of the *priA300* PriB$^+$ strains are the same as shown in Figure 5A and are included for comparison. The strains used are from left to right: GTN1867, GTN1713, GTN381, GTN1717, GTN1789, GTN1811.

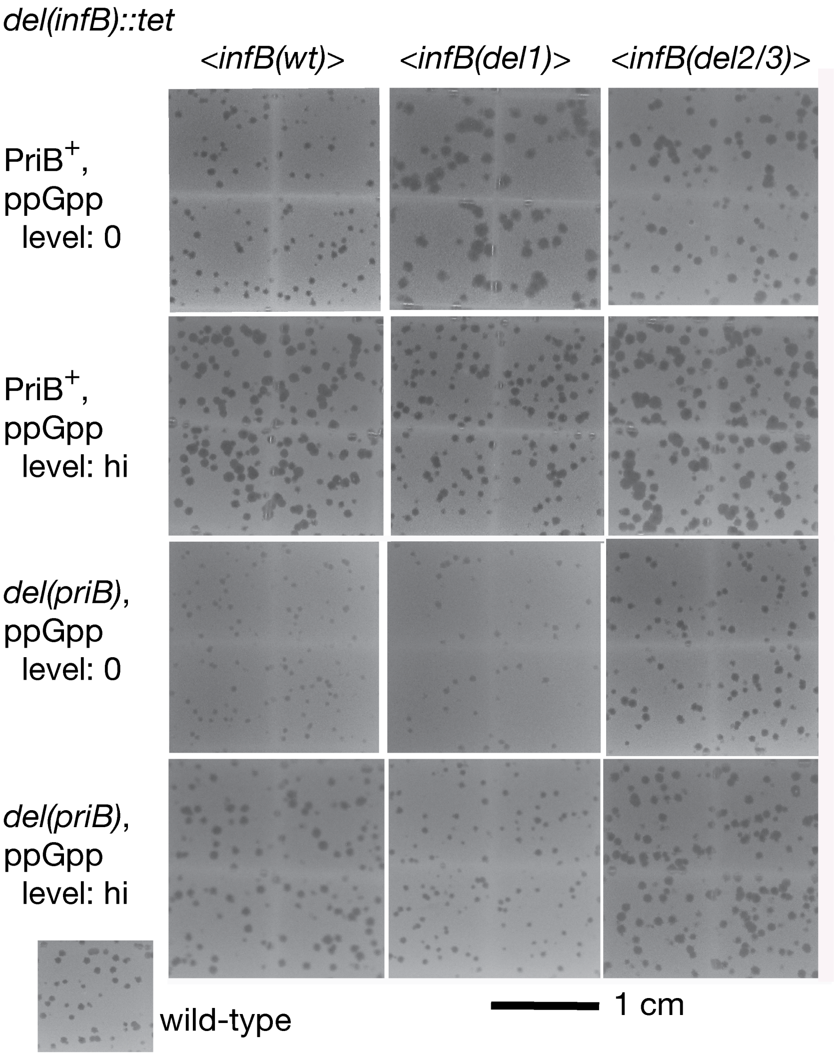

Figure S4. **Expression of IF2-1 or IF2-2/3 alone and their effect on Mu replication in the PriB$^+$ and *del(priB)* genetic backgrounds.** The effect of the *infB* alleles on the formation of Mu plaques at 0 and high ppGpp levels is shown. Approximately 4000 plaque-forming units of Mu were plated with the bacterial strains on a 100 x 15 mm petri plate, which was incubated overnight at 37°C. The specific indicator strains used (listed in ascending ppGpp order for each group) are PriB+ ppGpp0: GTN1700, GTN1701, GTN1702; PriB+ ppGpp hi: GTN1703, GTN1704, GTN1705; *del(priB)* ppGpp0: GTN1498, GTN1537, GTN1499; *del(priB)* ppGpp hi: GTN1502, GTN1503, GTN1505. The strain indicated as wild-type is GTN932, the standard strain used to titer Mu. The bar is a reference for 1 cm length.

Table S1. ***Escherichia coli* strains.**

Strain	Relevant genotypes	Source
Sources of mutant alleles:		
CF1693[a]	*del(relA)251::kan del(spoT)207::cat*	M. Cashel (Xiao *et al.*, 1991)
CF10237[b]	*del(relA)256 del(spoT)212*	M. Cashel (Harinarayanan *et al.*, 2008)
CF12271[c]	*del(relA)256 spoT202 zib563::Tn10*	M. Cashel (Sarubbi *et al.*, 1988)
CF12275[c]	*del(relA)256 spoT203 zib563::Tn10*	M. Cashel (Sarubbi et al., 1988)
CF5834[d]	*pyrE60 zib563::Tn10*	M. Cashel
NK5992[e]	*argA81::Tn10*	CGSC[f]
SS97[g]	*priA300 sulA::*Mu*d(lac,Ap,B::Tn9)*	S. Sandler (Sandler, 2000)
JC19272[h]	*del(priB)302 priC303::kan dnaC809*	S. Sandler (Sandler *et al.*, 1999)
JW0456-1[i]	*del(priC)752::kan*	CGSC (Baba *et al.*, 2006)
JW3140-5[j]	*del(argG)781::kan*	CGSC (Baba et al., 2006)
GTN932 constructs with the natural *infB(wt)* locus:		
GTN932	*del(gpt-lac)5*	(Madison *et al.*, 2012)
GTN381	GTN932 *priA300*	(Madison *et al.*, 2012)
GTN387	GTN932 *priC303::kan*	(GTN932)[k,l]
GTN394	GTN932 *del(priB)302*	(Madison *et al.*, 2012)

GTN699	GTN932 *priC303::kan del(relA)256*	(GTN387)[m]
GTN700	GTN932 *del(priB)302 del(relA)256*	(GTN394)[m]
GTN701	GTN932 *del(relA)256*	(GTN932)[m]
GTN702	GTN932 *priC303::kan del(relA)256 del(spoT)207::cat*	(GTN699)[n]
GTN703	GTN932 *del(priB)302 del(relA)256 del(spoT)207::cat*	(GTN700)[n]
GTN704	GTN932 *del(relA)256 del(spoT)207::cat*	(GTN701)[n]
GTN722	GTN932 *del(relA)256 spoT202 zib563::Tn10*	(GTN704)[o]
GTN723	GTN932 *del(relA)256 spoT203 zib563::Tn10*	(GTN704)[o]
GTN724	GTN932 *del(priB)302 del(relA)256 spoT202 zib563::Tn10*	(GTN703)[o]
GTN726	GTN932 *del(priB)302 del(relA)256 spoT203 zib563::Tn10*	(GTN703)[o]
GTN1486	GTN932 pBAD43 *del(priB)302 del(relA)256 del(spoT)207::cat*	(GTN703)[p]
GTN1487	GTN932 pBAD43 *del(priB)302 del(relA)256 spoT202 zib563::Tn10*	(GTN724)[p]
GTN1613	GTN932 *priA300 del(relA)251::kan*	(GTN381)[q]
GTN1617	GTN932 *priA300 del(relA)251::kan spoT202 zib563::Tn10*	(GTN1613)[o]
GTN1713	GTN932 *priA300 del(relA)256*	(GTN701)[r]
GTN1717	GTN932 *priA300 del(relA)256 spoT202 zib563::Tn10*	(GTN1713)[o]
GTN1765	GTN932 *del(priC)752::kan del(relA)256 spoT202 zib563::Tn10*	(GTN722)[s]
GTN1789	GTN932 *priA300 del(priB)302 del(relA)256*	(GTN1713)[t]
GTN1811	GTN932 *priA300 del(priB)302 del(relA)256 spoT202 zib563::Tn10*	(GTN1717)[t]
GTN1821	GTN932 *del(priC)752::kan*	(GTN932)[s]
GTN1829	GTN932 *del(relA)256 del(priC)752::kan*	(GTN701)[s]
GTN1867	GTN932 *priA300 del(relA)256 del(spoT)207::cat*	(GTN1713)[n]
GTN1872	GTN932 *del(priC)752::kan del(relA)256 spoT203 zib563::Tn10*	(GTN723)[s]
GTN1896	GTN932 *del(priC)752::kan del(relA)256 del(spoT)207::cat*	(GTN704)[s]

GTN932 *<nusAinfB(wt)cat> del(infB)1::tet* (GTN1050) constructs:

GTN1050	GTN932 *<nusAinfB(wt)cat> del(infB)1::tet*	(Madison *et al*., 2012)
GTN1122	GTN1050 *del(priB)302*	(GTN394)[u]
GTN1298	GTN1050 *priA300*	(Madison *et al*., 2012)
GTN1461	GTN1050 *del(argG)781::kan*	(GTN1050)[v]
GTN1498	GTN1050 *del(priB)302 del(relA)256 del(spoT)212 del(argG)781::kan*	(GTN703)[w]

GTN1502	GTN1050 *del(priB)302 del(relA)256 spoT202 del(argG)781::kan zib563::Tn10*	(GTN724)[x]
GTN1700	GTN1050 *del(relA)256 del(spoT)212 del(argG)781::kan*	(GTN704)[w]
GTN1703	GTN1050 *del(relA)256 spoT202 del(argG)781::kan zib563::Tn10*	(GTN722)[x]
GTN932 *<nusAinfB(del1)cat> del(infB)1::tet* (GTN1114 constructs):		
GTN1114	GTN932 *<nusAinfB(del1)cat> del(infB)1::tet*	(Madison *et al*., 2012)
GTN1117	GTN1114 *del(priB)302*	(Madison *et al*., 2012)
GTN1323	GTN1114 *priA300*	(Madison *et al*., 2012)
GTN1503	GTN1114 *del(priB)302 del(relA)256 spoT202 del(argG)781::kan zib563::Tn10*	(GTN724)[x]
GTN1537	GTN1114 *del(priB)302 del(relA)256 del(spoT)212 del(argG)781::kan*	(GTN703)[w]
GTN1701	GTN1114 *del(relA)256 del(spoT)212 del(argG)781::kan*	(GTN704)[w]
GTN1704	GTN1114 *del(relA)256 spoT202 del(argG)781::kan zib563::Tn10*	(GTN722)[y]
GTN1714	GTN1114 *priA300 del(relA)251::kan*	(GTN1323)[q]
GTN932 *<nusAinfB(del2/3)cat> del(infB)1::tet* (GTN1115) constructs:		
GTN1115	GTN932 *<nusAinfB(del2/3)cat> del(infB)1::tet*	(Madison *et al*., 2012)
GTN1119	GTN1115 *del(priB)302*	(Madison *et al*., 2012)
GTN1297	GTN1115 *priA300*	(Madison *et al*., 2012)
GTN1499	GTN1115 *del(priB)302 del(relA)256 del(spoT)212 del(argG)781::kan*	(GTN703)[w]
GTN1505	GTN1115 *del(priB)302 del(relA)256 spoT202 del(argG)781::kan zib563::Tn10*	(GTN724)[y]
GTN1593	GTN1115 pBAD43 *del(priB)302 del(relA)256 spoT202 del(argG)781::kan zib563::Tn10*	(GTN1505)[p]
GTN1594	GTN1115 pSPC*nusAinfB(del1) del(priB)302 del(relA)256 spoT202 del(argG)781::kan zib563::Tn10*	(GTN1505)[p]

GTN1596	GTN1115 pBAD43 *del(priB)302 del(relA)256 del(spoT)212 del(argG)781::kan*	(GTN1499)^p
GTN1597	GTN1115 pSPC*nusAinfB(del1) del(priB)302 del(relA)256 del(spoT)212 del(argG)781::kan*	(GTN1499)^p
GTN1702	GTN1115 *del(relA)256 del(spoT)212 del(argG)781::kan*	(GTN704)^w
GTN1705	GTN1115 *del(relA)256 spoT202 del(argG)781::kan*	(GTN722)^y
GTN1711	GTN1115 *priA300 del(relA)251::kan*	(GTN1297)^q

GTN932 pSPC*nusAinfB(wt, del1, or del2/3) del(infB)1::tet del(priB)302* constructs:

GTN1466	GTN932 pSPC*nusAinfB(wt) del(infB)1::tet del(priB)302 del(relA)256 spoT202 del(argG)781::kan zib563::Tn10*	(GTN724)^z
GTN1480	GTN932 pSPC*nusAinfB(wt) del(infB)1::tet del(priB)302 del(relA)256 del(spoT)207::cat del(argG)781::kan*	(GTN703)^z
GTN1539	GTN1480 but with pSPC*nusAinfB(del2/3)*	(GTN703)^z
GTN1541	GTN1466 but with pSPC*nusAinfB(del1)*	(GTN724)^z
GTN1543	GTN1466 but with pSPC*nusAinfB(del2/3)*	(GTN724)^z
GTN1610	GTN1480 but with pSPC*nusAinfB(del1)*	(GTN703)^z

GTN932 pSPC*nusAinfB(del1, or del2/3) del(infB)1::tet* (PriB$^+$) constructs:

GTN1675	GTN932 pSPC*nusAinfB(del2/3) del(infB)1::tet del(relA)256 del(spoT)207::cat del(argG)781::kan*	(GTN704)^z
GTN1679	GTN932 pSPC*nusAinfB(del1) del(infB)1::tet del(relA)256 spoT202 del(argG)781::kan zib563::Tn10*	(GTN722)^z
GTN1681	GTN1679 but with pSPC*nusAinfB(del2/3)*	(GTN722)^z
GTN1698	GTN1675 but with pSPC*nusAinfB(del1)*	(GTN704)^z
GTN1943	GTN932 pSPC*nusAinfB(del1) del(infB)1::tet del(relA)256 del(argG)781::kan*	(GTN701)^z
GTN1957	GTN1943 but with pSPC*nusAinfB(del2/3)*	(GTN701)^z

GTN932 pSPC*nusAinfB(del1, or del2/3) del(infB)1::tet priA300 del(relA)256 constructs:*

GTN1757	GTN932 pSPC*nusAinfB(del2/3) del(infB)1::tet priA300 del(relA)256 del(argG)781::kan*	(GTN1713)^z
GTN1759	GTN1757 *spoT202 zib563::Tn10*	(GTN1717)^z
GTN1800	GTN1757 *del(spoT)207::cat*	(GTN1759)^n
GTN1856	GTN1759 but with pSPC*nusAinfB(del1)*	(GTN1717)^z
GTN1858	GTN1757 but with pSPC*nusAinfB(del1)*	(GTN1713)^z
GTN1860	GTN1800 but with pSPC*nusAinfB(del1)*	(GTN1858)^n

[a] Source of *relA* and *spoT* deletions linked to kanamycin and chloramphenicol resistance, respectively.

[b] Source of markerless *relA* and *spoT* deletions introduced into strains by P1 transduction (see Notes d and e)

[c] Source of the *spoT202* or *spoT203* allele, introduced into strains by co-transduction with the tetracycline resistance marker (*zib563::Tn10).*

[d] Source of the *pyrE60* allele, introduced into strains by co-transduction with tetracycline resistance. This was in preparation for introduction of the *del(spoT212)* allele from CF10237 by co-transduction with *pyrE*$^+$. (Tetracycline-sensitive constructs were chosen for use.)

[e] Source of the *argA81* allele, introduced into strains by co-transduction with tetracycline resistance. This was in preparation for introduction of the *del(relA)* allele from CF10237 by co-transduction with *argA*$^+$.

[f] The Coli Genetic Stock Center, Yale University

[g] Source of the *priA300* allele*,* introduced into strains as previously described (Madison *et al.*, 2012).

[h] Source of *priC303::kan* and the markerless *del(priB)302* alleles introduced into strains as previously described (Madison et al., 2012).

[i] Source of the *del(priC)752::kan* allele, introduced by P1 transduction.

[j] The source of the *del(argG)781::kan* allele, used for introduction of the *del(infB)1::tet* allele*,* whose tetracycline resistance is not sufficiently robust for efficient direct selection. The *infB* knockout was introduced either by co-transduction with *argG*$^+$ to a *del(argG)781::kan* strain or by co-transduction with *del(argG)781::kan*, selecting for kanamycin resistance.

[k] The parent strain that is the recipient strain for P1 transductions or for transformation with a plasmid is indicated in parenthesis.

[l] P1 transduction of the *priC303::kan* allele from JC19272, selecting for kanamycin resistance.

[m] The markerless *del(relA)*256 allele was introduced into strains by first transducing the *argA81::Tn10* from NK5992, selecting for tetracycline resistance. The resulting strain was transduced to ArgA$^+$ inherited from CF10237, screening for co-inheritance of the *del(relA)256* allele by the SMG test, described under Experimental Procedures.

[n] P1 transduction of *del(spoT)207::cat* from CF1693, selecting for chloramphenicol resistance.

[o] P1 transduction of *zib563::Tn10* from CF12271 (*spoT202*) or CF12275 (*spoT203*), selecting for tetracycline resistance and screening for coinheritance of *spoT202* or *spoT203*, indicated by chloramphenicol sensitivity (if the recipient strain is *del(spoT)207::cat*) or by a small colony phenotype. Inheritance of the *spoT* allele was verified by sequencing.

[p] The parent strain was transformed with the indicated plasmid, selecting for spectinomycin resistance.

[q] P1 transduction of the *del(relA)251::kan* allele from CF1693, selecting for kanamycin resistance.

[r] The *priA300* allele from SS97 was introduced as previous described (Madison *et al.*, 2012).

[s] P1 transduction of *del(priC)752::kan* from JW0456-1, selecting for kanamycin resistance.

[t] The *del(priB)302* allele was introduced from JC19272 as previously described (Sandler *et al.*, 1999).

[u] The *<nusAinfB(wt)cat>, <nusAinfB(del1)cat>*, or *<nusAinfB(del2/3)cat>* from GTN1050, GTN1114, or GTN1115, respectively, was introduced by P1 transduction, selecting for chloramphenicol resistance. The *del(argG)781::kan* allele from JW3140-5 was then introduced, selecting for kanamycin resistance and screening for arginine auxotrophy. Finally, the strain was transduced to ArgG^{+}, inherited from GTN1050, screening for co-inheritance of *del(infB)1::tet.*

[v] The *del(argG)781::kan* allele from JW3140-5 was introduced by P1 transduction, screening for tetracycline resistance and the retention of the *del(infB)1::tet* allele.

[w] The construction was conducted in three stages: *i*) replacement of the *del(spoT)207::cat* allele with the markerless *del(spoT)212* allele; *ii*) introduction of *<nusAinfB(wt)cat>, <nusAinfB(del1)cat>*, or *<nusAinfB(del2/3)cat>* from GTN1050, GTN1114, or GTN1115, respectively; and *iii*) the *del(infB)::tet* allele was introduced. In the first stage, the *zib563::Tn10* was introduced by P1 transduction, selecting for tetracycline resistance and screening for co-inheritance of *pyrE60* (pyrimidine auxotrophy). The resulting strain was transduced to PyrE^{+}, inherited from CF10237. Transductants were screened for loss of chloramphenicol resistance, and the *del(spoT)212* allele was confirmed by PCR analysis. In the second stage, the *<nusAinfBcat>* allele was introduced by P1 transduction, selecting for chloramphenicol resistance. In the third stage, the *del(argG)781::kan* from GTN1461 was transduced, selecting for tetracycline resistance and screening for co-inheritance of the *del(infB)::tet* allele. Tetracycline resistance from this allele is not robust enough for direct selection. The presence of the allele was also verified by PCR analysis described previously (Madison *et al.*, 2012).

[x] The *<nusAinfB(wt)cat>, <nusAinfB(del1)cat>*, or *<nusAinfB(del2/3)cat>* was introduced from GTN1050, GTN1114, or GTN1115, respectively, selecting for chloramphenicol resistance. The *del(infB)1::tet* was co-transduced with *del(argG)781::kan* from GTN1461 as described under note *w*.

[y] Same as note *u* except that the *del(infB)::tet* allele was introduced from GTN1461 by co-transduction with *del(argG)781::kan,* selected by kanamycin resistance.

[z] The parent strain was transformed with the indicated plasmid, selecting for spectinomycin resistance. The *del(infB)1::tet* allele from GTN1461 was introduced by co-transduction with *del(argG)781::kan*, selected by resistance to kanamycin.

SI References

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