**SUPPLEMENTAL MATERIAL**

# **Table S1** *Escherichia coli* K-12 strains used in this work

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Strains** | | ***recA*** | ***recN*** | **Other relevant genotype** | **Source or derivation** |
| GW5245 | | *938*::*cat* | + |  | (Marsh and Walker 1987) |
| N3071 | | + | + | *recB268*::Tn*10* | (Lloyd *et al*. 1987) |
|  | |  |  |  |  |
| **AB1157 background***a* | | | | | |
| AB1157 | + | | + |  | (Dewitt and Adelberg 1962) |
| JC7623 | + | | + | *recB21 recC22 sbcB15 sbcC201* | (Kushner *et al.* 1971; Horii and Clark 1973; Lloyd and Buckman 1985) |
| SS12613 | + | | *∆100*::*kan* | *recB21 recC22 sbcB15 sbcC201* | (Warr *et al.* 2019) |
| SS12978 | *∆100*::*kan* | | + | *recB21 recC22 sbcB15 sbcC201* | SS4952 → JC7623*f* |
| SS12991 | *4190* | | + | *ygaD1*::*kan recB21 recC22 sbcB15 sbcC201* | SS12986 → JC7623*f* |
| SS13448 | *4190* | | *∆100*::*kan* | *zfj-3131*::Tn*10 ygaD1*::*kan recB21 recC22 sbcB15 sbcC201* | SS12989 → SS12613*g* |
| SS13458 | + | | *∆200*::*frt* | *recB21 recC22 sbcB15 sbcC201* | Flipped version of SS12613 (pCP20) |
| SS13465 | *4190* | | *∆200*::*frt* | *ygaD1*::*kan recB21 recC22 sbcB15 sbcC201* | SS12986 → SS13458*f* |
| SS13499 | *4155,4190,4136*:: *gfp-918* (A206E) | | + | *ygaD1*::*kan recAo1403 recB21 recC22 sbcB15 sbcC201* | SS13493 → JC7623*f* |
| SS13709 | + | | + | *gal-76*::Tn*10 ∆attB*::*sulAp-gfp recB21 recC22 sbcB15 sbcC201* | SS1465 → JC7623*g* |
| SS13710 | + | | *∆100*::*kan* | *gal-76*::Tn*10 ∆attB*::*sulAp-gfp recB21 recC22 sbcB15 sbcC201* | SS1465 → SS12613*g* |
| SS13711 | *4190* | | + | *ygaD1*::*kan gal-76*::Tn*10 ∆attB*::*sulAp-gfp recB21 recC22 sbcB15 sbcC201* | SS1465 → SS12991*g* |
| SS13712 | *∆100*::*kan* | | + | *gal-76*::Tn*10 ∆attB*::*sulAp-gfp recB21 recC22 sbcB15 sbcC201* | SS4952 → SS13709*f* |
| SS13719 | + | | *∆200*::*frt* | *gal-76*::Tn*10 ∆attB*::*sulAp-gfp recB21 recC22 sbcB15 sbcC201* | SS1465 → SS13458*g* |
| SS13720 | *4190* | | *∆200*::*frt* | *ygaD1*::*kan gal-76*::Tn*10 ∆(attB)*::*sulAp-gfp recB21 recC22 sbcB15 sbcC201* | SS12986 → SS13719*f* |
| SS13721 | *4155,4136*::*gfp-918* (A206E) | | + | *ygaD1*::*kan recAo1403 recB21 recC22 sbcB15 sbcC201* | SS11644 → JC7623*f* |
| SS13723 | *4155,4136*::*gfp-918* (A206E) | | *∆200*::*frt* | *zfj-3131*::Tn*10 ygaD1*::*kan recAo1403 recB21 recC22 sbcB15 sbcC201* | SS11770 → SS13458*f* |
| SS13724 | *4155,4190,4136*:: *gfp-918* (A206E) | | *∆200*::*frt* | *zfj-3131*::Tn*10 ygaD1*::*kan recAo1403 recB21 recC22 sbcB15 sbcC201* | SS13704 → SS13458*f* |
|  |  | |  |  |  |
| **BW25113 background***b* | | | | | |
| BW25113 | | *+* | *+* |  | (Datsenko and Wanner 2000; Baba *et al.* 2006) |
| SS4952 | | *∆100*::*kan* | *+* |  | Lab stock (Baba *et al.* 2006) |
|  | |  |  |  |  |
| **JC13509 background***c* | | | | | |
| JC13509 | | + | + |  | (Sandler *et al.* 1996) |
| SS1465 | | + | + | *gal-76*::Tn*10 ∆attB*:: *sulAp-gfp* | Lab stock |
| SS6321 | | + | + | *hupA*::*mCherry FRT ∆attB*::*sulAp-gfp* | (Bhattacharyya *et al.* 2014) |
| SS7117 | | + | + | *∆galK200*::*frt ∆attB*::*sulAp-mCherry* | (Warr *et al.* 2019) |
| SS9510 | | *∆100*::*kan* | + | *hupA*::*mCherry FRT ∆attB*::*sulAp-gfp* | Lab stock |
| SS9988 | | + | *∆100*::*kan* |  | (Warr *et al.* 2019) |
| SS10168 | | *∆200*::*frt* | + | *malE*::Tn*10-9 (cam) lexA3* | Lab stock |
| SS10191 | | *∆200*::*frt* | + | pKD46 *malE*::Tn*10-9 (cam) lexA3* | Lab stock |
| SS10360 | | + | *∆100*::*kan* | *hupA*::*mCherry FRT ∆attB*::*sulAp-gfp* | (Warr *et al.* 2019) |
| SS11644 | | *4155,4136*::*gfp-918* (A206E) | + | *ygaD1*::*kan recAo1403 ∆galK200*::*frt ∆attB*::*sulAp-mCherry* | Lab stock (Renzette *et al.* 2005) |
| SS11770 | | *4155,4136*::*gfp-918* (A206E) | + | *zfj-3131*::Tn*10 ygaD1*::*kan recAo1403 ∆galK200*::*frt ∆attB*::*sulAp-mCherry* | Lab stock |
| SS12173 | | *∆200*::*frt* | + | *lexA3 malE*::Tn*10 ∆galK200*::*frt  ∆attB*::*sulAp-mCherry* | Lab stock |
| SS12334 | | + | *∆100*::*kan* | *∆galK200*::*frt ∆attB*::*sulAp-mCherry* | (Warr *et al.* 2019) |
| SS12683 | | + | *∆100*::*kan* | *pheA18*::Tn*10* | CAG12158 → SS9988*g* |
| SS12943 | | *4155,4136*::*gfp-918* (A206E) | *∆100*::*kan* | *zfj-3131*::Tn*10 ygaD1*::*kan recAo1403 ∆galK200*::*frt ∆attB*::*sulAp-mCherry* | SS11770 → SS12334*g* |
| SS12954 | | *∆200*::*frt* | + | pAK11 [SS10168] | This study*h* |
| SS12986 | | *4190* | + | *ygaD1*::*kan malE*::Tn*10-9 (cam) lexA3* | This study*i* |
| SS12990 | | *4190* | + | *ygaD1*::*kan hupA*::*mCherry FRT  ∆attB*::*sulAp-gfp* | SS12986 → SS6321*f* |
| SS13426 | | + | *∆200*::*frt* | *hupA*::*mCherry FRT ∆attB*::*sulAp-gfp* | Flipped version of SS10360 (pCP20) |
| SS13459 | | *4190* | *∆200*::*frt* | *ygaD1*::*kan hupA*::*mCherry FRT  ∆attB*::*sulAp-gfp* | SS12986 → SS13426*f* |
| SS13491 | | *∆200*::*frt* | + | pAK18 [SS12173] | This study*h* |
| SS13493 | | *4155,4190,4136*::*gfp-918* (A206E) | + | *ygaD1*::*kan recAo1403 malE*::Tn*10-9 (cam) lexA3* | This study*i* |
| SS13497 | | *4155,4190,4136*::*gfp-918* (A206E) | + | *ygaD1*::*kan recAo1403 ∆galK200*::*frt  ∆attB*::*sulAp-mCherry* | SS13493 → SS7117*f* |
| SS13725 | | *4155,4190,4136*::*gfp-918* (A206E*)* | *∆200*::*frt* | *zfj-3131 ygaD1*::*kan recAo1403 ∆galK200*::*frt ∆attB*::*sulAp-mCherry* | SS13704 → SS12334*g* |
|  | | | | | |
| **KL227 background***d* | | | | | |
| KL227 | | + | + |  | (Singer *et al.* 1989) |
| CAG5052 | | + | + | *metB1btuB3191*::Tn*10* | (Singer *et al.* 1989) |
|  | |  |  |  |  |
| **MG1655 background***e* | | | | | |
| MG1655 | | + | + |  | (Guyer *et al.* 1981; Jensen 1993; Blattner *et al.* 1997) |
| APS121 | | + | + | *∆cynX*::I-*Sce*ICS*-kan ∆attB*::PBADI-*Sce*I | (Meddows *et al.* 2004) |
| CAG12158 | | + | + | *pheA18*::Tn*10* | (Singer *et al.* 1989) |
| CAG18642 | | + | + | *zfj-3131*::Tn*10* | (Singer *et al.* 1989) |
| SS2989 | | + | + | *∆cynX*::I-*Sce*ICS*-kan ∆attB*::PBADI-*Sce*I *recB268*::Tn*10* | Lab stock (Meddows *et al.* 2005) |
| SS12684 | | + | + | *pheA18*::Tn*10 ∆cynX*::I-*Sce*ICS*-kan  ∆attB*::PBADI-*Sce*I | CAG12158 → APS121*g* |
| SS12685 | | + | *∆100*::*kan* | *pheA18*::Tn*10 ∆cynX*::I-*Sce*ICS*-kan  ∆attB*::PBADI-*Sce*I | SS12683 → APS121*g* |
| SS12857 | | *938*::*cat* | + | *∆cynX*::I-*Sce*ICS*-kan ∆attB*::PBADI-*Sce*I | Lab stock |
| SS12989 | | *4190* | + | *zfj-3131*::Tn*10 ygaD1*::*kan* | SS12986 → CAG18642*f* |
| SS13453 | | *4190* | + | *zfj-3131*::Tn*10 ygaD1*::*kan ∆cynX*::I-*Sce*ICS*-kan ∆attB*::PBADI-*Sce*I | SS12989 → APS121*g* |
| SS13457 | | + | *∆100*::*kan* | *∆cynX*::I-*Sce*ICS*-kan ∆attB*::PBADI-*Sce*I | SS9988 → SS12685*f* |
| SS13463 | | *4190* | *∆100*::*kan* | *zfj-3131*::Tn*10 ygaD1*::*kan ∆cynX*::I-*Sce*ICS*-kan ∆attB*::PBADI-*Sce*I | SS12989 → SS13457*g* |
| SS13704 | | *4155,4190,4136*::*gfp-918* (A206E) | *+* | *zfj-3131*::Tn*10 ygaD1*::*kan recAo1403* | SS13493 → CAG18642*g* |

*a* AB1157 strain has the following genotype: F– *thr-1 araC14 leuB6*(Am) ∆*(gpt-proA)62 lacY1 tsx-33 qsr'-0 glnX44*(AS) *galK2*(Oc) *λ*– *Rac-0 hisG4*(Oc) *rfbC1 mgl-51 rpoS396*(Am) *rpsL31*(strR) *kdgK51 xylA5, mtl-1 argE3*(Oc) *thiE1*.

*b* BW25113 is derived from BD792 strain and has the following genotype: F– Δ(*araD-araB*)*567* Δ*lacZ4787*(::*rrnB-3*) *λ*– *rph-1* Δ(*rhaD-rhaB*)*568 hsdR514*.

*c* JC13509 is derived from SK362 strain and has the following genotype: F– *lacMS286* Φ*80dIIlacBK1 sulB103 argE4 his-4 thi-1 xyl-5 mtl-1* SmR T6R. The *lacMS286* Φ*80dIIlacBK1* codes for two partial non-overlapping deletions of the lac operon (Konrad 1977; Zieg and Kushner 1977).

*d*KL227 has the following genotype: Hfr *λ*– *relA1 spoT1 metB1.*

*e* MG1655 stain has the following genotype: F– *λ*– *rph-1*.

*f* Select for kanamycin resistance and then screen by PCR.

*g* Select for tetracycline resistance and then screen by PCR.

*h*Select for ampicillin and kanamycin resistance. Screen by PCR and DNA sequence analysis.

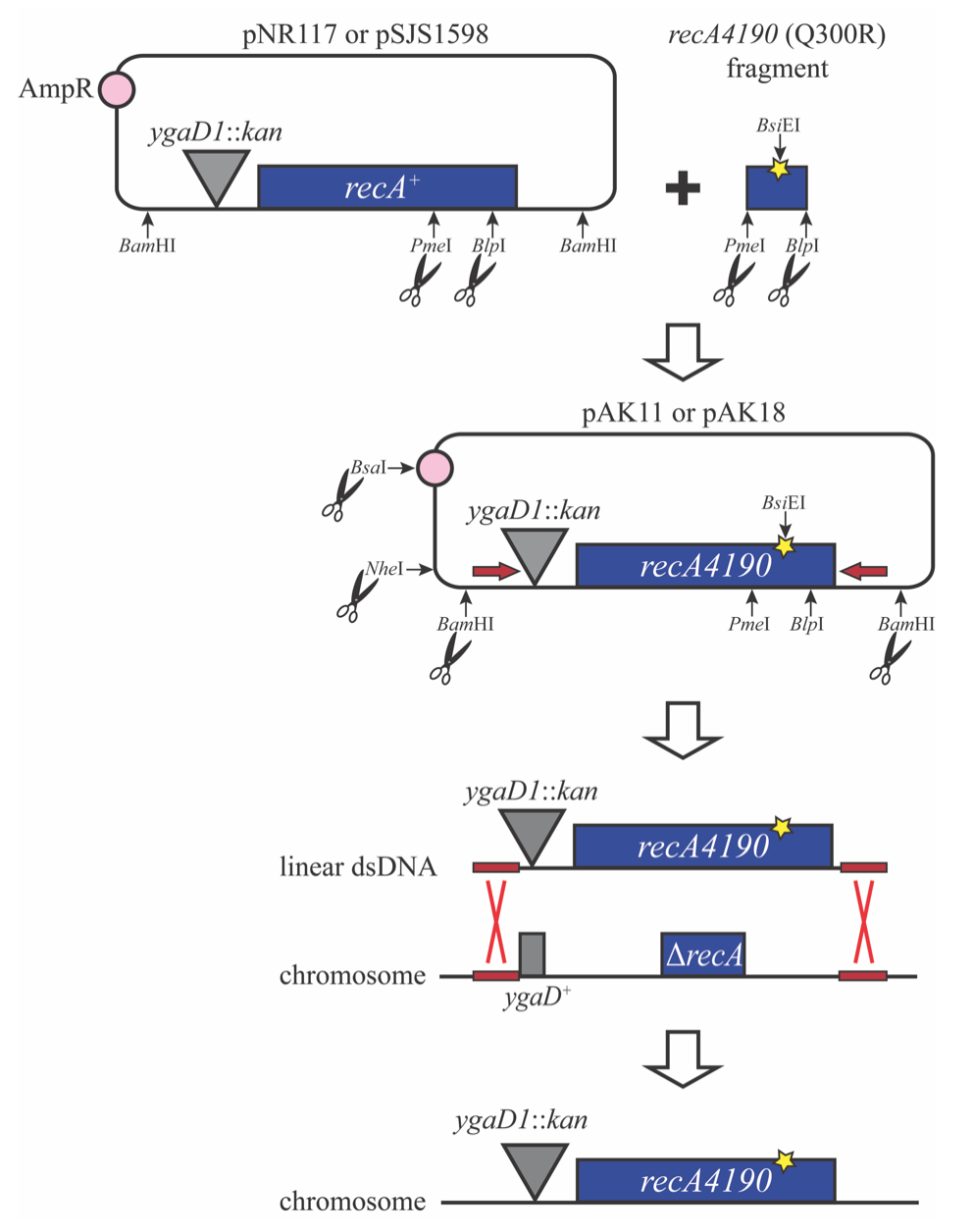
*i* See Materials and Methods section for details on strain construction.

# **Table S2** Oligonucleotide primers used in this work

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence (5’ to 3’)** | **Specificity** |
| prSJS468 | AATCGCTGCGCCGTTTAAACAGGC | Forward primer in *recA* overlapping *Pme*I site |
| prSJS507 | TTTGCCACTGCCCGCGGTGAAGG | Forward primer in *ygaD* upstream of *recA* |
| prSJS563 | GCGTACCGCACGATCCAACAGGCG | Reverse primer in *recX* downstream of *recA* |
| prSJS853 | GTGGTATGCGTTACACCTTGAGTGC | Forward primer in *ybhC* near the λ attachment site |
| prSJS854 | GGCACTGGGTAGTTGTTAACTTACC | Reverse primer in *ybhB* near the λ attachment site |
| prSJS1090 | TCGTCGTTGACTCCGTGGCGGCACTGACGCCG | Forward primer upstream of *recA* K250 |
| prSJS1091 | AAAATCTTCGTTAGTTTCTGCTACGCCTTCGC | Reverse primer downstream of *recA* K250 |
| prSJS1100 | CGCTATCATCTACAGAGAAATCCGGCGTTGAGTTCGGG | Reverse primer in *recA* just after *Blp*I site |
| prSJS1378 | TACCATCTGAATCTGATTCATCCG | Forward primer upstream of *recN* |
| prSJS1379 | TTCGGGCCCGAGCAGTACGTGGC | Reverse primer downstream of *recN* |
| prSJS1594 | GCCCGAGCAGTACGTGGCTCATCTGTAATATGCCGATGATAATAGACCTTTCATCACTTTAAAACCTTTTGCTTTCCGGTCTTACGG | Reverse primer downstream of *recN* |
| pSJS1655A | GCAACGCCAACACCATCTTCCTGACG | Forward primer upstream of *Bsi*WI site in *ygaD* in front of *recA* |
| pSJS1656A | CCATAATCGGTGCCGCGAGTTTACGTCG | Reverse primer in *recX* downstream of *recA* |

# **Table S3** Plasmids used in this work

|  |  |  |
| --- | --- | --- |
| **Plasmid** | **Description** | **Reference** |
| pAK11 | pBR322 derivative with a *Bam*HI fragment containing *ygaD1*::*kan recA4190* (Q300R) with upstream and downstream chromosomal sequences, AmpR, KanR | This study |
| pAK18 | pBR322 derivative with a *Bam*HI fragment containing *ygaD1*::*kan recAo1403 recA4155,4190,4136*::*gfp‐918* (A206E) with upstream and downstream chromosomal sequences, AmpR, KanR | This study |
| pCP20 | expression of Flp recombinase, temperature sensitive replication, AmpR, CamR | (Cherepanov and Wackernagel 1995) |
| pKD46 | expression of the λ Red system under control of arabinose-inducible promoter, temperature sensitive replication, AmpR | (Datsenko and Wanner 2000) |
| pNR117 | pBR322 derivative with a *Bam*HI fragment containing *ygaD1*::*kan recA*+ with upstream and downstream chromosomal sequences, AmpR, KanR | Lab stock |
| pSJS1598 | pBR322 derivative with a *Bam*HI fragment containing *ygaD1*::*kan recAo1403 recA4155,4136*::*gfp‐918* (A206E) with upstream and downstream chromosomal sequences, AmpR, KanR | Lab stock |



# **Figure S1.** Construction of the *recA4190* (Q300R) mutant. A *recA4190* gene fragment carrying a novel *Bsi*EI site was ligated with either pNR117 or pSJS1598 vector treated with PmeI and BlpI restriction enzymes. Resulting plasmids were designated as pAK11 and pAK18, respectively. Presence of the *recA4190* (Q300R) mutation was verified by PCR and DNA sequencing. The pAK11 plasmid was further linearized by digestion with BamHI and BsaI, while the pAK18 plasmid was subjected to an additional digestion with NheI, which was followed by PCR amplification with primers prSJS1655A and prSJS1656A. Generated linear DNAs were recombined onto the chromosome of a strain carrying ∆*recA* via λ Red-mediated recombination as described elsewhere (Datsenko and Wanner 2000). Recombination that occurred between homologous arms highlighted in red lead to creation of successful recombinants that were selected for resistance to kanamycin and screened by PCR and sequencing.

# **LITERATURE CITED**

Baba T., T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, *et al.*, 2006 Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. 2: 2006.0008. https://doi.org/10.1038/msb4100050

Bhattacharyya B., N. P. George, T. M. Thurmes, R. Zhou, N. Jani, *et al.*, 2014 Structural mechanisms of PriA-mediated DNA replication restart. Proc. Natl. Acad. Sci. 111: 1373–8. https://doi.org/10.1073/pnas.1318001111

Blattner F. R., G. Plunkett, C. A. Bloch, N. T. Perna, V. Burland, *et al.*, 1997 The complete genome sequence of *Escherichia coli* K-12. Science 277: 1453–1462. https://doi.org/10.1126/science.277.5331.1453

Cherepanov P. P., and W. Wackernagel, 1995 Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. Gene 158: 9–14. https://doi.org/10.1016/0378-1119(95)00193-a

Datsenko K. A., and B. L. Wanner, 2000 One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. U. S. A. 97: 6640–6645. https://doi.org/10.1073/pnas.120163297

Dewitt S. K., and E. A. Adelberg, 1962 The occurrence of a genetic transposition in a strain of *Escherichia coli*. Genetics 47: 577–585.

Guyer M. S., R. R. Reed, J. A. Steitz, and K. B. Low, 1981 Identification of a sex-factor-affinity site in *E. coli* as gamma delta. Cold Spring Harb. Symp. Quant. Biol. 45 Pt 1: 135–140. https://doi.org/10.1101/sqb.1981.045.01.022

Horii Z. I., and A. J. Clark, 1973 Genetic analysis of the recF pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. J. Mol. Biol. 80: 327–344. https://doi.org/10.1016/0022-2836(73)90176-9

Jensen K. F., 1993 The *Escherichia coli* K-12 “wild types” W3110 and MG1655 have an *rph* frameshift mutation that leads to pyrimidine starvation due to low *pyrE* expression levels. J. Bacteriol. 175: 3401–3407. https://doi.org/10.1128/jb.175.11.3401-3407.1993

Konrad E. B., 1977 Method for the isolation of *Escherichia coli* mutants with enhanced recombination between chromosomal duplications. J. Bacteriol. 130: 167–72.

Kushner S. R., H. Nagaishi, A. Templin, and A. J. Clark, 1971 Genetic recombination in *Escherichia coli*: the role of exonuclease I. Proc. Natl. Acad. Sci. 68: 824–827. https://doi.org/10.1073/pnas.68.4.824

Lloyd R. G., and C. Buckman, 1985 Identification and genetic analysis of *sbcC* mutations in commonly used *recBC* *sbcB* strains of *Escherichia coli* K-12. J. Bacteriol. 164: 836–44.

Lloyd R. G., C. Buckman, and F. E. Benson, 1987 Genetic analysis of conjugational recombination in *Escherichia coli* K12 strains deficient in RecBCD enzyme. J. Gen. Microbiol. 133: 2531–2538. https://doi.org/10.1099/00221287-133-9-2531

Marsh L., and G. C. Walker, 1987 New phenotypes associated with mucAB: alteration of a MucA sequence homologous to the LexA cleavage site. J. Bacteriol. 169: 1818–1823. https://doi.org/10.1128/jb.169.5.1818-1823.1987

Meddows T. R., A. P. Savory, and R. G. Lloyd, 2004 RecG helicase promotes DNA double-strand break repair. Mol. Microbiol. 52: 119–132. https://doi.org/10.1111/j.1365-2958.2003.03970.x

Meddows T. R., A. P. Savory, J. I. Grove, T. Moore, and R. G. Lloyd, 2005 RecN protein and transcription factor DksA combine to promote faithful recombinational repair of DNA double-strand breaks. Mol. Microbiol. 57: 97–110. https://doi.org/10.1111/j.1365-2958.2005.04677.x

Renzette N., N. Gumlaw, J. T. Nordman, M. Krieger, S.-P. Yeh, *et al.*, 2005 Localization of RecA in *Escherichia coli* K-12 using RecA-GFP. Mol. Microbiol. 57: 1074–1085. https://doi.org/10.1111/j.1365-2958.2005.04755.x

Sandler S. J., H. S. Samra, and A. J. Clark, 1996 Differential suppression of *priA2*::*kan* phenotypes in *Escherichia coli* K-12 by mutations in *priA*, *lexA*, and *dnaC*. Genetics 143: 5–13.

Singer M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, *et al.*, 1989 A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. 53: 1–24.

Warr A. R., A. N. Klimova, A. N. Nwaobasi, and S. J. Sandler, 2019 Protease-deficient SOS constitutive cells have RecN-dependent cell division phenotypes. Mol. Microbiol. 111: 405–422. https://doi.org/10.1111/mmi.14162

Zieg J., and S. R. Kushner, 1977 Analysis of genetic recombination between two partially deleted lactose operons of *Escherichia coli* K-12. J. Bacteriol. 131: 123–132.