**Table S1.** Strains used in this study.

| Strain | Genotype | Reference |
| --- | --- | --- |
| HM1 | trpC2, pheA1 | |
| HM313 | recA-mGFPmut2-spec | Simmons, *Journal of Bacteriology*, 2009 |
| HM209 | trpC2, pheA1, ΔrecA-MLS/CAT | Cheo, *Biochimie*, 1992 |
| HM410 | ΔrecO-CAT | Simmons, *Journal of Bacteriology*, 2009 |
| BKE25280 | trpC2, ΔrecU-MLS | Bacillus Genetic Stock Center Koo BM et al. (In preparation) |
| HM430 | trpC2, pheA1, ΔaddAB-Kan | Million-Weaver, PNAS, 2015 |
| BKE22310 | trpC2, ΔrecO-MLS | Bacillus Genetic Stock Center Koo BM et al. (In preparation) |
| HM211 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] MLS$^R$ | Merrikh, *Nature*, 2011 |
| HM352 | trpC2, pheA1, thrC::Pxis-lacZ, ICEBs1(0) [head-on] MLS$^R$, recA-mGFPmut2-specR | Million-Weaver, PNAS, 2015 |
| HM 624 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] MLS$^R$ | Merrikh, Nature, 2011 |
| HM630 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] MLS$^R$, recA-mGFPmut2-spec | Million-Weaver, PNAS, 2015 |
| HM640 | trpC2, pheA1, thrC::Pxis-lacZ [co-directional] MLS$^R$, ICEBs1(0) | Million-Weaver, PNAS, 2015 |
| HM639 | trpC2, pheA1, thrC::Pxis-lacZ [co-directional] MLS$^R$ | Million-Weaver, PNAS, 2015 |
| HM824 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on], ΔrecA-MLS/CAT | Million-Weaver, PNAS, 2015 |
| HM825 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] MLS$^R$, ΔrecA-MLS/CAT | This study |
| HM907 | trpC2, pheA1, thrC::Pxis-lacZ [co-directional] MLS$^R$, ICEBs1(0), ΔrecA-MLS/CAT | This study |
| HM908 | trpC2, pheA1, thrC::Pxis-lacZ [co-directional] MLS$^R$, ΔrecA-MLS/CAT | This study |
| HM765 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on], MLS$^R$, ΔrecO-CAT | This study |
| HM676 | trpC2, pheA1, thrC::Pxis-lacZ [head-on], MLS$^R$, ΔrecO-CAT | This study |

| | | |
|---|---|---|
| HM766 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] , MLS$^R$, ΔrecO-CAT | This study |
| HM677 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] MLS$^R$ , recA-mGFPmut2-spec, ΔrecO-CAT | This study |
| HM857 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] , MLS$^R$, ΔaddAB-Kan | This study |
| HM760 | trpC2, pheA1, thrC::Pxis-lacZ, ICEBs1(0) [head-on] , MLS$^R$, recA-mGFPmut2-specR, ΔaddAB-Kan | This study |
| HM770 | trpC2, pheA1, thrC::Pxis-lacZ [head-on], MLS$^R$, ΔaddAB-Kan | This study |
| HM667 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] MLS$^R$ , recA-mGFPmut2-spec, ΔaddAB-Kan | This study |
| HM807 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] , MLS$^R$, ΔrecO-CAT, ΔaddAB-Kan | This study |
| HM854 | trpC2, pheA1, thrC::Pxis-lacZ, ICEBs1(0), MLS$^R$, recA-mGFPmut2-specR, ΔrecO-CAT, ΔaddAB-Kan | This study |
| HM808 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] , MLS$^R$, ΔrecO-CAT, ΔaddAB-Kan | This study |
| HM817 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] MLS$^R$ , recA-mGFPmut2-spec, ΔrecO-CAT, ΔaddAB-Kan | This study |
| HM871 | trpC2, pheA1, thrC::Pxis-lacZ [co-directional] MLS$^R$ , ICEBs1(0), ΔrecO-CAT, ΔaddAB-Kan | This study |
| HM872 | trpC2, pheA1, thrC::Pxis-lacZ [co-directional] MLS$^R$, ΔrecO-CAT, ΔaddAB-Kan | This study |
| HM1247 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] , MLS$^R$, ΔrecO-CAT, ΔaddAB-Kan, ΔrecA-MLS/CAT | This study |
| HM1246 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] MLS$^R$, ΔrecO-CAT, ΔaddAB-Kan, ΔrecA-MLS/CAT | This study |
| HM1069 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] , MLS$^R$, ΔrecU | This study |
| HM1076 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] , MLS$^R$, ΔrecU | This study |
| HM1146 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] , MLS$^R$, ΔrecU, ΔrecA-MLS/CAT | This study |
| HM1155 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] , MLS$^R$, ΔrecU, ΔrecA-MLS/CAT | This study |
| HM1231 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] , MLS$^R$, ΔaddAB-Kan, ΔrecU | This study |
| HM1090 | trpC2, pheA1, thrC::Pxis-lacZ [head-on], MLS$^R$, ΔaddAB-Kan, ΔrecU | This study |
| HM1288 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] , MLS$^R$, ΔaddAB-Kan, ΔrecU, ΔrecA-MLS/CAT | This study |
| HM1287 | trpC2, pheA1, thrC::Pxis-lacZ [head-on], MLS$^R$, ΔaddAB-Kan, | This study |

| | ΔrecU, ΔrecA-MLS/CAT | |
|---|---|---|
| HM1085 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] , MLS$^R$, ΔrecO-CAT, ΔrecU | This study |
| HM1089 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] , MLS$^R$, ΔrecO-CAT, ΔrecU | This study |
| HM1304 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] , MLS$^R$, ΔrecO-CAT, ΔrecU, ΔrecA-MLS/CAT | This study |
| HM1302 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] , MLS$^R$, ΔrecO-CAT, ΔrecU, ΔrecA-MLS/CAT | This study |
| HM1305 | trpC2, pheA1, thrC::Pxis-lacZ ICEBs1(0) [head-on] , MLS$^R$, ΔrecO-CAT, ΔrecU, ΔaddAB-Kan | This study |
| HM1138 | trpC2, pheA1, thrC::Pxis-lacZ [head-on] , MLS$^R$, ΔrecO-CAT, ΔrecU, ΔaddAB-Kan | This study |

**Supplemental Figure 1**
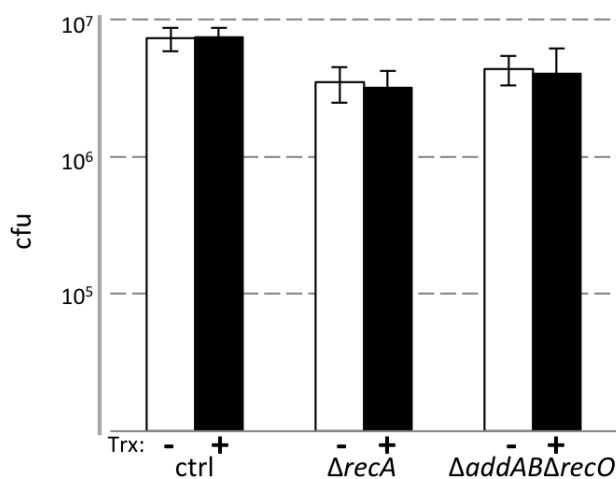


**A)** Colony forming units arising from exponentially growing cultures at $OD_{600}$=0.3 were enumerated in the presence (Trx +, black bars) and absence (Trx -, white bars) of transcription from $P_{xis}$-*lacZ* for strains harboring the construct in the co-directional orientation.

**Supplemental Figure 2**



**A. RNAP ChIP**

**B. DnaC ChIP**
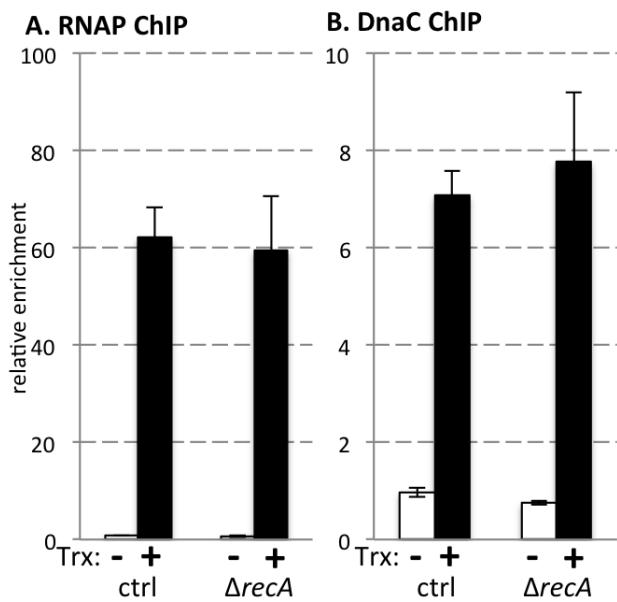
**A)** Relative association of RNA polymerase with the head-on gene-containing region. **B)** Relative association of the replicative helicase, DnaC, with the head-on gene-containing region. Data shown represents averages of 3 biological replicates. Error bars represent standard errors of the means.