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# Supplemental Data

# Transposition into Replicating DNA Occurs through Interaction with the Processivity Factor

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

| ***E. coli* Strains** | **Genotype** | **Reference** |
|---|---|---|
| MC4100 | *araD139 Δ(argF-lac)169 rpsL150 relA1 flhD5301 deoC1 ptsF25 rbsR22 e14⁻ Δ(fimB-fimE)632::IS1 Δ(fruK-yeiR)725* | (Casadaban, 1976) |
| NLC28 | MC4100 $Val^R$ | (McKown et al., 1988) |
| NLC51 | NLC28 $Val^R$ *recA56* | (McKown et al., 1988) |
| BB101 | B F- *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm ΔslyD* (DE3) | (Chivers and Sauer, 1999) |
| BLR21(DE3) | BL21(DE3) *srl::*Tn*10 ΔrecA* | Mark Sutton |
| SY2 | *Δlac X74 rpsL araD139 Δ(ara-leu)7697 galU galK hsr⁻ hsm⁺sulA::lacZ'YA*::$Kan^R$ | (Ohmori et al., 1995) |
| JP1386 | NLC28 *Δara714* | (Peters and Craig, 2001) |

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|---|---|---|
| AP330 | JP1386 *sulA::lacZ'YA*::Kan$^R$ | JP1386 X (P1)SY2 |
| AP389 | JP1386 *sulA::lacZ'YA*::frt::Cm$^R$::*frt* | AP330 X λRED "recombineered" with PCR product JEP191/JEP192 (Datsenko and Wanner, 2000) |
| AP427 | JP1386 *sulA::lacZ'YA*::*frt* | Cam$^R$ cassette removed from AP389 by expression of FLP recombinase from pCP20 (Cherepanov and Wackernagel, 1995) |
| STL3607 | AB1157 *recF400*::Kan$^R$ | (Lovett et al., 2002) |
| AP461 | AP427 *recF400*::Kan$^R$ | AP 427 X (P1) STL3607 |
| ***Yeast* Strains** | **Genotype** | **Reference** |
| EGY40 [pSH18-34] | MATa ura3-52 trp1-1 leu2-3,112 [pSH18-34] | (Liachko and Tye, 2005) |

**Table S2. Primers used in this study**

| Primer name | Primer sequence | Use |
|---|---|---|
| JEP3 | 5' - ATT TTC GTA TTA GCT TAC GAC GCT ACA CCC - 3' | Left end Tn7 primer for mapping transposition |
| JEP4 | 5' - ACT TTA TTG TCA TAG TTT AGA TCT ATT TTG - 3' | Right end Tn7 primer for mapping transposition |
| JEP129 | 5' – CGG GTG AGG GAC ATT ACA GT – 3' | Amplicon for cloning β |
| JEP130 | 5' – ATG AAA TTT ACC GTA GAA CGT GAG CA – 3' | Amplicon for cloning β |
| JEP149 | 5' – TTA ATT AAA AAT AAT GAC CGA GCT AGC TCT AAC GCT GGG – 3' | Introducing the Q122A mutation into TnsE |
| JEP150 | 5' – TTA ATT AAA AAT AAT GAC CGA GCT AGC TCT GCC TGT GGG – 3' | Introducing the L123A mutation into TnsE |
| JEP151 | 5' – TTA ATT AAA AAT AAT GAC CGA GCT GCC TCT AAC TGT GGG – 3' | Introducing the L125A mutation into TnsE |
| JEP152 | 5' – TTA ATT AAA AAT GCT GAC CGA GCT AGC TCT AAC TGT GGG – 3' | Introducing the L129A mutation into TnsE |
| JEP153 | 5' – TTA ATT AAA GCT AAT GAC CGA GCT AGC TCT AAC TGT GGG – 3' | Introducing the F130A mutation into TnsE |
| JEP155 | 5' – CAG AAA TAG GAG TTA ATT GCA AAT AAT GAC CGA GCT AG – 3' | Introducing the L131A mutation into TnsE |
| JEP191 | 5'- AGA AAA ACT CAT CGA GCA TCA AAT GAA ATGAAA CTG CAA TTT ATT CAT A GT GTA | Amplicon for replacing the $Kan^R$ cassette with a *frt*-$Cam^R$- |

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| | GGC TGG AGC TGC TTC-3’ | *frt* cassette |
| JEP192 | 5’-ATG AGC CAT ATT CAA CGG GAA ACG TCT TGC TCG AGG CCGCGA TTA AAT TCA TAT GAA TAT CCT CCT TAG -3’ | Amplicon for replacing the $Kan^R$ cassette with a *frt*-$Cam^R$-*frt* cassette |
| JEP272 | 5’- GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTC GAA GGA GAT AGA ACC ATG GTT AGG CTA GCT ACA TTT AAT GAC – 3’ | TnsE fusions for use in the two hybrid assay |
| JEP273 | 5’- GGGG AC CAC TTT GTA CAA GAA AGC TGG GTT TTA ATG CGT AAA TTG CTC TC – 3’ | TnsE fusions for use in the two hybrid assay |
| JEP275 | 5’- GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTC GAA GGA GAT AGA ACC ATG AAA TTT ACC GTA GAA CG – 3’ | β fusions for use in the two hybrid assay |
| JEP247 | 5’ – AAT CTC CCT CCC ACA AGC AGT AAC - 3’ | Construction of pGAP |
| JEP248 | 5’ – CCT CAG CAA TGC TTT CAC CAC CTC AGC TAT CCG CGG TAT TCC AGA CGA - 3’ | Construction of pGAP |
| JEP249 | 5’ – GCT GAG GTG GTG AAA GCA TTG CTG AGG AGC TGA AAC AAG GCG GGA CTC – 3’ | Construction of pGAP |
| JEP250 | 5’ – CGC GCA CCA GAG AAG AAC CC -3’ | Construction of pGAP |
| JEP345 | 5’ – GCA ATG CTT TCA CCA CCT CA -3’ | Complementary to the removed 20-nt ssDNA upon making the gap |
| JEP278 | 5’ - GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTC GAA GGA GAT AGA ACC ATG AAT CGG TTG TAC CCG GAA C – 3’ | δ fusions for use in the two hybrid assay |

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| JEP279 | 5’ - GGGG AC CAC TTT GTA CAA GAA AGC TGG GTT TCA ACC GTC GAT AAA TAC GTC – 3’ | δ fusions for use in the two hybrid assay |
| JEP286 | 5’ – AGG TTG TTG TAC AGA ATA TGA TTC GGT TGT ACC CGG AAC – 3’ | Construction of pTYB12-δ |
| JEP265 | 5’ – TAA AGA TCT CGA GTC AAC CGT CGA TAA ATA CG– 3’ | Construction of pTYB12-δ |
| JEP266 | 5’ – GGT GGT CTC GAG TTA AAG ATG AGG AAC CGG– 3’ | Construction of pTYB12-δ’ |
| JEP287 | 5’ – CCC ACT TGT ACA GAA TAT AGA TGG TAT CCA TGG TTA CG– 3’ | Construction of pTYB12-δ’ |
| JEP268 | 5’ – CCT TCC TGT ACA GAA TGC TGG TCA TAT GAG TTA TCA GGT CTT AGC CCG– 3’ | Construction of pTYB12-γ |
| JEP269 | 5’ – GCG GCT CTC GAG TCA CTC CTT TTT TGC TTT GGT TGC TCC– 3’ | Construction of pTYB12-γ |

**Table S3. Plasmids used in this study**

| Name | Relevant information |
|---|---|
| pGPS2.1 | Mini-Tn7::Cam$^R$ containing plasmid with a conditional origin of replication. (NEB) |
| pBTM$^{gw}$ | pBTM16 derivative containing a gateway cassette, 2μ, LEU2, GAD4-AD (Liachko and Tye, 2005) |
| pGAD$^{gw}$ | pGAD2F derivative containing a gateway cassette, 2μ, TRP1, LEXA-DBD (Liachko and Tye, 2005) |
| pBAD24 | pBR322 derivative containing a multi-cloning site under the control of an arabinose promoter, ampicillin resistant cloning vector (Guzman et al., 1995) |
| pTA106 | pSC101 replicon, ampicillin resistant cloning vector |
| pβHK | a pET16b (Novagen) derivative containing a protein kinase motif and $His_6$ tag fused to the N-terminus of β, ampicillin resistant (Kelman et al., 1995) |
| pCP20 | Temperature sensitive plasmid with thermal induction of FLP recombinase (Cherepanov and Wackernagel, 1995) |
| pKD46 | Temperature sensitive plasmid with the λ Red proteins under arabinose control (Datsenko and Wanner, 2000) |
| pKD3 | Plasmid encoding ampicillin resistance that allows PCR amplification of a gene cassette encoding chloramphenicol resistance flanked by *frt* sites recognized by the FLP recombinase (Datsenko and Wanner, 2000) |
| pCAW11 | pET22b (Novagen) derivative encoding TnsE, ampicillin resistant (Wolkow et al., 1996) |
| pCW4 | pACYC184 derivative encoding TnsABCDE, tetracycline resistant (Waddell and Craig, 1988) |

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| pCW4mm76 | pACYC184 derivative encoding TnsABCDE with a miniMu insertion in the TnsE gene, tetracycline resistant (Waddell and Craig, 1988) (For data presented in text regarding TnsD-mediated transposition with *dnaN* overexpression) |
| pCW15 | pACYC184 derivative encoding TnsABC, chloramphenicol resistant (Waddell and Craig, 1988) (For data shown in Figure 5.A.) |
| pJRC210 | pBBR322 derived plasmid encoding *dnaN* (Sutton, 2005)(For data presented in text regarding TnsD-mediated transposition with *dnaN* overexpression) |
| pJP104 | pTA106 derivative encoding TnsE, ampicillin resistant (Peters and Craig, 2001)(For data shown in Figure 3a) |
| pJP131 | pBAD24 derivative encoding TnsE, ampicillin resistant (Peters and Craig, 2001)(For data shown in Figure 7) |
| pQS100 | pTA106 vector encoding TnsABC constructed by cloning the 4919 bp of *Pvu* II fragment encoding TnsABC from pCW4 into the *Sma* I site of pTA106 with the *tnsA* gene proximal to the vector *Hind* III site. (Shi et al., 2008)(For data shown in Figure 5.B.) |
| pQS102 | pTA106 vector encoding TnsABC+E constructed by cloning the 4919 bp of *Pvu* II fragment encoding TnsABC from pCW4 into the *Sma* I site of pJP104 vector with the *tnsC* gene proximal to the vector *Hind* III site. (Shi et al., 2008)(For data shown in Figure 5.B.) |
| pARP30 | Plasmid pGAD with *tnsE* fused to the yeast transcription activation domain. Gateway clone using PCR product from primers JEP272 and JEP273 and pJP131 as template. (For data shown in Figure 2 and 4.A.). |
| pARP35 | Plasmid pBTM with $\beta$ fused to the GAL4 binding domain. Gateway clone |

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| | using PCR product from primers JEP275 and JEP276 and *E. coli* chromosome (NLC28) as template. (For data shown in Figure 2 and 4.A.) |
| pARP36 | Plasmid pGAD with *holA* fused to the yeast transcription activation domain. Gateway clone using PCR product from primers JEP278 and JEP279 and *E. coli* chromosome (NLC28) as template. (For data shown in Figure 2). |
| pAP8 | Plasmid pGEM-T containing the *dnaN* gene. |
| pARP63 | pACYC184 *dnaN* gene constructed by cloning an *Eag* I fragment from pAP8 into the *Eag* I site within the $Tet^R$ gene of pACYC184. The direction of β transcription is the same as that of the $Tet^R$ gene. (For data shown in Figure 5.B.) |
| pAP42 | pTYB12 containing *holB* fused to the Sce VMA intein tag and a chitin binding domain for affinity purification and subsequent tag cleavage of the δ' protein. A PCR product from primers JEP287 and JEP267 was digested with *BsrG* I and *Xho* I and cloned into pTYB12 (NEB). |
| pAP43 | pTYB12 containing HolA fused to the to the Sce VMA intein tag and a chitin binding domain for affinity purification and subsequent tag cleavage of the δ protein. A PCR product from primers JEP286 and JEP265 was digested with *BsrG* I and *Xho* I and cloned into pTYB12 (NEB). |
| pAP44 | pTYB12 containing the γ encoding portion of the *dnaX* gene fused to the Sce VMA intein tag and a chitin binding domain for affinity purification and subsequent tag cleavage of the γ protein. A PCR product from primers JEP268 and JEP269 was digested with *BsrG* I and *Xho* I and cloned into pTYB12 (NEB). A stop codon was introduced at amino acid position 431 and the terminal amino acid was changed to glutamate to produce the γ |

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| | protein alone and not τ. |
| pGEM-*attTn7* | pGEM-T cloning vector containing the *attTn7* locus (Finn et al., 2007). |
| pGAP | pGEM-T containing *attTn7* from *E. coli*, and two *Nb. BbvC* I recognition sequences separated by 20 bp. An overlapping PCR product generated by using primer pairs JEP247/JEP248 and JEP249/JEP250 digested with *Ava* I and *Nhe* I replaces the ~200bp *Ava* I-*Nhe* I fragment of pGEM-*attTn7* (Finn et al., 2007). This vector is used to make the target DNA with single-stranded gap (Figure 6). |

**Table S4**. Sequences used in computational analysis (Parks and Peters, 2007).

| **Host Species** | **Accession #** |
|---|---|
| *E.coli* | CAA35687 |
| *P.stuartii* | ABG21684 |
| *S. putrefaciens 200* | EAY53112 |
| *I. loihiensis L2TR* | AA83441 |
| *S. baltica OS155* | ABN63817 |
| *S. putrefaciens CN-32.2* | ABP77648 |
| *S. putrefaciens CN-32.1* | ZP_00814471 |
| *S. loihica PV-4* | ABO25690 |
| *P. carbinolicus* | YP_358326 |
| *H. chejuensis KCTC2396* | YP_438100 |
| *A. ferrooxidans* | AAC21663 |
| *B. cereus ATCC10987* | AAS39122 |
| *Staphylococcus sp.693-2* | ABG49263 |
| Immobile Ac/T-DNA vector pNU400 | ABB59986.1 |
| Tn917 transposase sequence | AF061336 |

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