Supplementary Data NAR-00675 D-2009 R2

Covo et al, 2009. Translesion DNA Synthesis-Assisted Non-Homologous End-Joining of Complex Double-Strand Breaks Prevents Loss of DNA Sequences in Mammalian Cells

Table S1. Repair of complex DSB (Substrate LP41) relative to simple DSB (Substrate LP40) in hamster cells defective in NHEJ genes

Cell type/vector		Number of colonies (DNA amount [b])		Relative Repair [a] %
		Kan^R	Cm^R	
CHO K1 (wild-type)				56.1±7.0
	LP41	203 (350)	253 (24)	
	LP40	393 (350)	260 (24)	
	LP41	228 (350)	229 (24)	
	LP40	194 (350)	99 (24)	
	LP41	37 (350)	101 (24)	
	LP40	87 (350)	158 (24)	
	LP41	220 (350)	588 (24)	
	LP40	374 (350)	634 (24)	
	LP41	204 (350)	252 (24)	
	LP40	344 (350)	221 (24)	
	LP41	99 (350)	280 (24)	
	LP40	433 (350)	632 (24)	
CHO XR1 (*XrccIV*$^{-/-}$)				NA
	LP41	6 (350)	636 (24)	
	LP40	2 (350)	364 (24)	
	LP41	0 (350)	76 (24)	
	LP40	1 (350)	96 (24)	
	LP41	6 (350)	233 (24)	
	LP40	1 (350)	364 (24)	
CHO XRS5 (*Ku80*$^{-/-}$)				51.0±4.9
	LP41	106 (200)	1228 (24)	
	LP40	203 (200)	1122 (24)	
	LP41	18 (200)	348 (24)	
	LP40	35 (200)	330 (24)	
	LP41	58 (200)	572 (24)	
	LP40	121 (200)	676 (24)	

NHEJ assays were performed with the indicated cell lines as described in the legend to Table 1 and under Materials and Methods. The relative repair represents the average obtained from the experiments shown for each cell type. [a] Relative repair of cDSB relative to sDSB.

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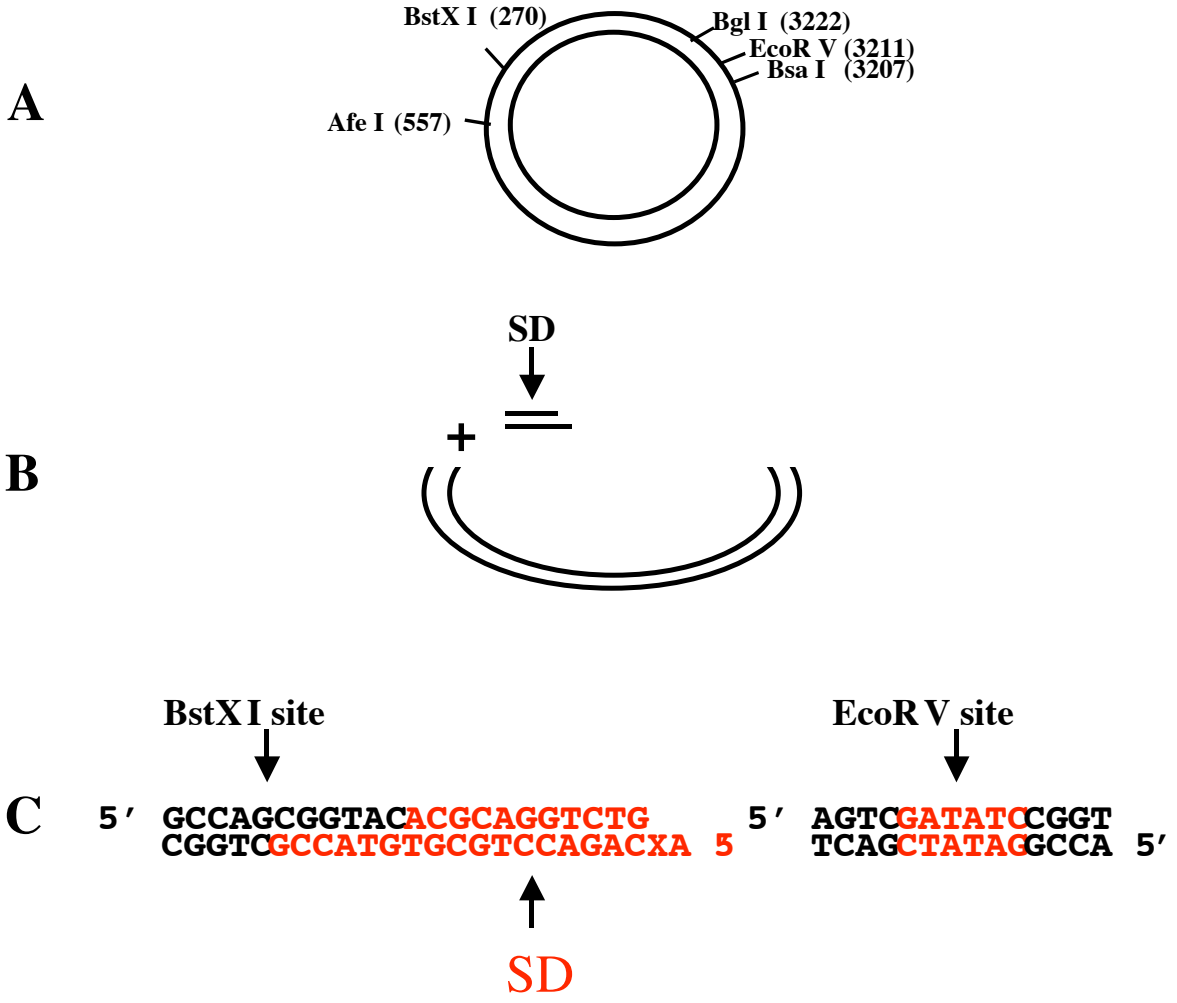

Figure S1. Construction of linear plasmids LP40 and LP41. (**A**) Map of parental plasmid pSKSL annotated with relevant restriction sites. The EcoRV site was cloned into plasmid pSKSL between the BglI and BsaI sites. In order to obtain the linear vector fragment needed for the construction of LP41 and LP40 the plasmid was digested with BstXI and BglI. (**B**) Ligation of the short duplex (SD) oligonucleotide to the BstxI site ("left" side of the vector). (**C**) The cDSB generated after ligation of the SD (in red) to the vector. X marks the abasic site modification. The "right" side of the DSB was created by digestion with EcoRV.

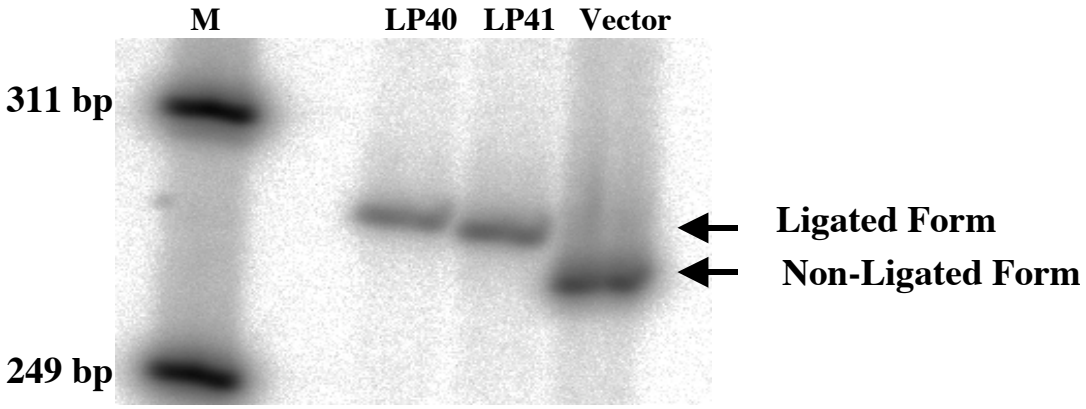

Figure S2. Confirmation of ligation efficiency of LP41/40. Samples from the ligation mixture and a control non-ligated vector were digested with AfeI (see Fig. 1S), radiolabled at their 5' using polynucleotide kinase and ATPγ^{32}P, and fractionated by a native 6% PAGE. Arrows indicate ligated and non-ligated forms, M, DNA size marker.

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K1 (wt)

sDSB

(13)	GCCAGCGGTACACGCAGGTCTG*T*TATCCGGTATCATTGCAGCACTGGGG		[0]
(3)	GCCAGCGGTACACGCAGGTCTG	ATCCGGTATCATTGCAGCACTGGGG	[-2]
(1)	GCCAGCGGTACACG	ATCCGGTATCATTGCAGCACTGGGG	[-10]
(1)	GCCAGCGGTACACGCAGGTCT	*G*GGG	[-22]
(2)		*T*GCAGCACTGGGG	[-56]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-196]
(1)	TAGAAGCATATCTGCAATATTACAAAAATAGCCTTAATTTGTAACAATA		[Hyb]

cDSB

(1)	GCCAGCGGTACACGCAGGTCTG*T*TATCCGGTATCATTGCAGCACTGGGG		[0]
(4)	GCCAGCGGTACACGCAGGTCTG	ATCCGGTATCATTGCAGCACTGGGG	[-2]
(1)	GCCAGCGGTACACGCAGGTCT	ATCCGGTATCATTGCAGCACTGGGG	[-3]
(2)	GCCAGCGGTACACGCAGGTC	ATCCGGTATCATTGCAGCACTGGGG	[-4]
(1)	GCCAGCGGTACACGCAG	ATCCGGTATCATTGCAGCACTGGGG	[-7]
(1)	GCCAGCGGTACACGCAGGTCT	*G*TATCATTGCAGCACTGGGG	[-8]
(1)	GCCAGCG	ATCCGGTATCATTGCAGCACTGGGG	[-16]
(1)	GCCAG	*C*GGTATCATTGCAGCACTGGGG	[-22]
(1)	GCCAGCGGTACACGCAGGT	*C*TGGGG	[-23]
(1)	GCCA	*GC*AGCACTGGGG	[-38]
(1)		*AC*TGGGG	[-53]
(4)	GTGTTCGATCCGAAAGGCTGGGCGCTGTTCCGTTCCTTCAAAGCCGTCAA		[Hyb]

Xrs5 (*Ku80*$^{-/-}$)

sDSB

(1)	GCCAGCGGTACACGCGCAGGTCTG*T*TATCCGGTATCATTGCAGCACTGGGG		[0]
(1)	GCCAGCGGTACACGCGCAGGTCT	*GT*ATCATTGCAGCACTGGGG	[-8]
(1)	GCCAGCGGTACACGCGC	*A*TCCGGTATCATTGCAGCACTGGGG	[-8]
(1)	GCCAGCGGTACACGCGCAGG	*TC*ATTGCAGCACTGGGG	[-14]
(1)	GCCAGCGGTACACGCGCAGGTC	*T*TGCAGCACTGGGG	[-15]
(1)	GCCAGCGGTACACGCGC	TGCAGCACTGGGG	[-21]
(1)	GCCAGCGGTACA	TCATTGCAGCACTGGGG	[-22]
(1)	GCCAGCGGTA	*CA*TTGCAGCACTGGGG	[-23]
(1)	GCCAGCGGTACAC	GCAGCACTGGGG	[-24]
(5)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-77 to -196]
(1)	CCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCT		[+64 (pUC18)]

cDSB

(0)	GCCAGCGGTACACGCAGGTCTG*T*TATCCGGTATCATTGCAGCACTGGGG		[0]
(1)	GCCAGCGGTACACGCAGGTC	ATTGCAGCACTGGGG	[-13]
(1)	GCCAGCGGTACACGCAGGTCTG	*TT*GCAGCACTGGGG	[-13]
(1)	GCCAGCGGTACACGC	TTGCAGCACTGGGG	[-20]
(1)	GCCAGCGGTACAC	*GC*AGCACTGGGG	[-24]
(1)	GCCAGCGGTACACGCA	*GG*GG	[-28]
(6)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-54 to -112]
(3)	GGCTAGAGGAGAGAGACACACTTCTGGCTAAGATGTGTCACAAAGCTTCC		[Hyb]

Figure S3. DNA sequence analysis of plasmids with sDSB or cDSB that had underwent repair in CHO K1 wild-type, and CHO Xrs5 Ku80$^{-/-}$ cells. The numbers in the parentheses on the left show the number of occurrences. The numbers in the parentheses on the right, represent the length of the deletions or insertions at the original break point. The stretch of Δ indicates long deleted sequences. Hyb indicates hybrids of the linear plasmid and pUC18. Underlined nucleotides in Italics show the microhomology between the 2 ends. The site of the abasic site is at the second nucleotide to the left of the arrow (in Italics).

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A Repair of cDSB in Guetel cells (*Artemis*$^{-/-}$)

(0)	GCCAGCGGTACACGCAGGTCTG*T*TATCCGGTATCATTGCAGCACTGGGG		[0]
(4)	GCCAGCGGTACACGCAGGTCTGT	ATCCGGTATCATTGCAGCACTGGGG	[-1]
(1)	GCCAGCGGTACACGCAGGTCTGG	ATCCGGTATCATTGCAGCACTGGGG	[-1]
(6)	GCCAGCGGTACACGCAGGTCTG	ATCCGGTATCATTGCAGCACTGGGG	[-2]
(1)	GCCAGCGGTACACGCAGGTCTG	CCGGTATCATTGCAGCACTGGGG	[-4]
(1)	GCCAGCGGTACACGCAGGTCT	*G*GTATCATTGCAGCACTGGGG	[-8]
(1)	GCCAGCGGTACACGCAGGTCT	*G*TATCATTGCAGCACTGGGG	[-8]
(1)	GCCAGCGGTACACGCAGGT	*C*CGGTATCATTGCAGCACTGGGG	[-8]
(1)	GCCAGCGGTACACGCA	*GGT*ATCATTGCAGCACTGGGG	[-12]
(1)	GCCAGCG	ATCCGGTATCATTGCAGCACTGGGG	[-17]
(1)	GCCAGCGGTACACGCAGGT	*C*ACTGGGG	[-18]
(1)	GCCAGCGGTA	*CA*TTGCAGCACTGGGG	[-23]
(1)	G	*CC*GGTATCATTGCAGCACTGGGG	[-25]
(1)	GCCAGCGGTACACGCA	*GG*	[-23]
(1)		*A*TCCGGTATCATTGCAGCACTGGGG	[-26]
(1)	GCCAGCCCT	CAGCACTGGGG	[-29]
(1)	GCCAGCGGTACACG		[-46]
(1)	GCCAGC		[-48]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-77]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-81]
(1)	GCCAGCGGTACACGCAGGTCTGAAATCGGTCTCGCGGTATCATTGCAGC		[+10]

Total 28 seq

B Repair of cDSB in Guetel/DA4 cells (Artemis-proficient))

(0)	GCCAGCGGTACACGCAGGTCTG*T*TATCCGGTATCATTGCAGCACTGGGG		[0]
(4)	GCCAGCGGTACACGCAGGTCTGT	ATCCGGTATCATTGCAGCACTGGGG	[-1]
(6)	GCCAGCGGTACACGCAGGTCTG	ATCCGGTATCATTGCAGCACTGGGG	[-2]
(2)	GCCAGCGGTACACGCAGGTC	ATCCGGTATCATTGCAGCACTGGGG	[-4]
(1)	GCCAGCGGTACCC	CATTGCAGCACTGGGG	[-20]
(1)	GCCAGCGGTACA	*C*ATTGCAGCACTGGGG	[-21]
(1)	GCCAGC	*G*TATCATTGCAGCACTGGGG	[-23]
(1)		*C*CGGTATCATTGCAGCACTGGGG	[-25]
(1)	GCCAGC	CATTGCAGCACTGGGG	[-27]
(1)	GCCTGC		[-43]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-57]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-63]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-69]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-77]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-94]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-97]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-101]
(1)	ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ		[-115]
(1)	GCCAGCGGTACACGCAGGTCTGATCGGTCTCGATCATTGCAGCACTGGG		[0]*
(1)	GCCAGCGGTACACGCAGGTCTGACAATCGGTCTCGCGGTATCATTGCAG		[+8]

Total 28 seq

Figure S4. DNA sequence analysis of plasmids with cDSB that had underwent repair in human Guetel Artemis$^{-/-}$ cells or Guetel/DA4 Artemis-complemented cells. The numbers in the parentheses on the left show the number of occurrences. The numbers in the parentheses on the right, represent the length of the deletions or insertions at the original break point. The stretch of Δ indicates long deleted sequences. Hyb indicates hybrids of the linear plasmid and pUC18. Underlined nucleotides in Italics show the microhomology between the 2 ends. The site of the abasic site is at the second nucleotide to the left of the arrow (in Italics).

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A Repair of cDSB in CJ179 cells (*Artemis*$^{-/}$)$^{-}$

```
                                        ↓
(0) AACGCCAGCGGTACACGCAGGTCTGTTATCCGGTATCATTGCAGCACT [0]
(1) AACGCCAGCGGTACACGCAGGTCTGCAAATCCGGTATCATTGCAGCAC [+1]
(6) AACGCCAGCGGTACACGCAGGTCTGT ATCCGGTATCATTGCAGCACT [-1]
(2) AACGCCAGCGGTACACGCAGGTCTG  ATCCGGTATCATTGCAGCACT [-2]
(1) AACGCCAGCGGTACACGCAGGTCTG   TCCGGTATCATTGCAGCACT [-3]
(1) AACGCCAGCGGTACACGCAGGTCTG     CGGTATCATTGCAGCACT [-5]
(1) AACGCCAGCGGTACACGCAGGT             ATCATTGCAGCACT [-12]
(1) AACGCCAGCGGTAC            ATCCGGTATCATTGCAGCACT [-13]
(1) AACGCGCTGAACGCCAG            CGGTATCATTGCAGCACT [-14]
(1) AACG                       TCCGGTATCATTGCAGCACT [-23]
(1) AACGCCAGCGGT                         TTGCAGCACT [-25]
(1) AACGCCAGCGGTACACGCAGGTCTΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ [-25]
(1) ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ [-50]
(1) ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ [-97]
(5) AACGCCAGCGGTACACGCAGGTCTGAAATCCGGTAGTCGATATCCGTAT [hyb]
```

B Repair of cDSB in 48BR cells (*Artemis*$^{+/+}$)

```
                                        ↓
(0) AACGCCAGCGGTACACGCAGGTCTGTTATCCGGTATCATTGCAGCACT [0]
(4) AACGCCAGCGGTACACGCAGGTCTG TATCCGGTATCATTGCAGCACT [-1]
(8) AACGCCAGCGGTACACGCAGGTCTG  ATCCGGTATCATTGCAGCACT [-2]
(1) AACGCCAGCGGTACACGCAGGTC   ATCCGGTATCATTGCAGCACT [-4]
(1) AACGCCAGCGGTACACGCAGGTCTG     CGGTATCATTGCAGCACT [-5]
(1) AACGCCAGCGGTACACGCAGGTCT        GTATCATTGCAGCACT [-8]
(1) AACGCCAGCT                ATCCGGTATCATTGCAGCACT [-17]
(1) AACGCCAGC                 ATCCGGTATCATTGCAGCACT [-18]
(1) AACGCCAGCGGTACAC                        GCAGCACT [-23]
(1) AACGCCAGCGGT                       ATCATTGCAGCACT [-23]
(1)                                 GTATCATTGCAGCACT [-32]
(1) ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ [-250]
(3) GAACGCCAGCGGTACACAGCAGGTCTGATCCGGTATCATTGCAGCA   [hyb]
```

Figure S5. DNA sequence analysis of plasmids with cDSB that had underwent repair in human CJ179 Artemis$^{-/-}$ cells or 48BR Artemis-proficient cells. The numbers in the parentheses on the left show the number of occurrences. The numbers in the parentheses on the right, represent the length of the deletions or insertions at the original break point. The stretch of Δ indicates long deleted sequences. Hyb indicates hybrids of the linear plasmid and pUC18. Underlined nucleotides in Italics show the microhomology between the 2 ends. The site of the abasic site is at the second nucleotide to the left of the arrow (in Italics).