

SUPPLEMENTAL INFORMATION

DNA polymerases ζ and Rev1 mediate error-prone bypass of non-B DNA structures

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Table S1. Effect of *rev1* and *mms2* mutations on the spontaneous mutator phenotype of the *pol3-Y708A* strain.

Genotype	Can ^r mutation	
	Mutation rate (x10 ⁻⁸) ^a	Fold increase
Wild type	15 (13-19)	-
<i>rev1</i> Δ	25 (20-30)	1.6
<i>rev1-1</i>	14 (12-16)	0.9
<i>rev1-cd</i>	19 (14-28)	1.3
<i>pol3-Y708A</i>	140 (130-160)	9.3
<i>rev1</i> Δ <i>pol3-Y708A</i>	26 (24-29)	1.7
<i>rev1-1 pol3-Y708A</i>	47 (39-52)	3.1
<i>rev1-cd pol3-Y708A</i>	79 (68-97)	5.3
<i>mms2</i> Δ	87 (74-120)	5.8
<i>mms2</i> Δ <i>rev1-cd</i>	110 (91-130)	7.3
<i>mms2</i> Δ <i>pol3-Y708A</i>	250 (220-290)	17
<i>mms2</i> Δ <i>rev1-cd pol3-Y708A</i>	240 (200-300)	16

^aMutation rates are given as medians for at least nine independent cultures. 95% confidence limits are shown in parentheses.

Table S2. Rates for individual types of mutation in strains carrying mutant *pol3*, *rev3*, *rev1*, *mms2* alleles and/or their combinations.

Mutation	<i>pol3</i> -Y708A		<i>pol3</i> -Y708A <i>rev3</i> Δ		<i>pol3</i> -Y708A <i>rev1</i> Δ		<i>pol3</i> -Y708A <i>rev1</i> -1		<i>pol3</i> -Y708A <i>rev1</i> -cd		<i>mms2</i> Δ		<i>mms2</i> <i>pol3</i> -Y708A		<i>mms2</i> Δ <i>pol3</i> -Y708A <i>rev1</i> -cd	
	No. ^a	MR ^c ($\times 10^{-8}$)	No. ^a	MR ^d ($\times 10^{-8}$)	No. ^b	MR ^d ($\times 10^{-8}$)	No.	MR ^d ($\times 10^{-8}$)	No. ^b	MR ^d ($\times 10^{-8}$)	No. ^b	MR ^d ($\times 10^{-8}$)	No. ^b	MR ^d ($\times 10^{-8}$)	No. ^b	MR ^d ($\times 10^{-8}$)
Base substitutions																
GC \rightarrow AT	11	8.6	23	2.9	30	3.9	25	6.3	28	12	49	20	28	38	54	62
AT \rightarrow GC	8	6.2	7	0.88	5	0.65	11	2.8	3	1.3	9	3.6	6	8.1	6	6.9
GC \rightarrow TA	16	12	20	2.5	27	3.5	22	5.5	40	17	43	17	31	42	50	58
GC \rightarrow CG	55	43	0	<0.13	2	0.26	21	5.3	37	15	41	16	53	71	29	33
AT \rightarrow CG	7	5.4	0	<0.13	1	0.13	5	1.3	1	0.42	10	4.0	16	22	6	6.9
AT \rightarrow TA	29	23	36	4.5	60	7.8	48	12	39	16	17	6.8	13	17	25	29
Total	126	98	86	11	125	16	132	33	148	62	169	68	147	200	170	200
Small indels																
-1	8	6.2	4	0.50	4	0.52	16	4.0	5	2.1	35	14	18	24	12	14
-2	2	1.6	4	0.50	3	0.39	3	0.75	0	<0.42	1	0.40	0	<1.3	1	1.2
-3	0	<0.78	2	0.25	4	0.52	1	0.25	1	0.42	0	<0.40	0	<1.3	0	<1.2
-4	0	<0.78	2	0.25	0	<0.13	0	<0.25	0	<0.42	0	<0.40	1	1.3	0	<1.2
-5	0	<0.78	2	0.25	1	0.13	0	<0.25	0	<0.42	0	<0.40	0	<1.3	0	<1.2
+1	4	3.1	14	1.8	12	1.6	5	1.3	8	3.3	3	1.2	1	1.3	2	2.3
Total	14	11	28	3.5	24	3.1	25	6.3	14	5.9	39	16	20	27	15	17
Large rearrangements																
Deletions between short direct repeats	15	12	92	12	50	6.5	26	6.5	14	5.9	1	0.40	6	8.1	11	13
Other deletions	0	<0.78	1	0.13	1	0.13	0	<0.25	1	0.42	0	<0.40	0	<1.3	0	<1.2
Duplications	0	<0.78	1	0.13	0	<0.13	3	0.75	2	0.84	0	<0.40	0	<1.3	0	<1.2
Complex mutations^e																
Type I (≤ 6 nt)	24	19	1	0.13	0	<0.13	1	0.25	7	2.9	8	3.3	12	16	11	13
Type II (≥ 7 nt)	0	<0.78	4	0.50	0	<0.13	0	<0.25	2	0.84	0	<0.40	1	1.3	1	1.2
Other mutations^f																
	1	0.78	1	0.13	0	<0.13	1	0.25	1	0.42	0	<0.40	0	<1.3	0	<1.2
Total	180^c	140	214	27	200^c	26	188^c	47	189	79	217^c	87	186	250	208	240

^aData for the *pol3*-Y708A and *pol3*-Y708A *rev3* Δ strains are from Ref. 22.

^bFull mutational spectra are shown in Fig. S5.

^cTwo *can1* mutants of the *pol3*-Y708A strain, one of the *pol3*-Y708A *rev1* Δ strain, one of the *pol3*-Y708A *rev1*-1 strain and four of the *mms2* Δ strain carried double point mutations. The mutations were separated by 93, 476, 156, 15, 18, 278, 944 and 484 nucleotides, respectively. These were counted as eight individual mutations.

^dRate for each type of mutation was calculated as follows: $MR_i = (M_i/M_T) \times MR$, where M_i is the number of mutations of the particular type, M_T is the total number of mutations, and MR is the rate of Can^r mutation in the corresponding strain determined by fluctuation analysis.

^eComplex mutations type I are defined as replacements of one to six adjacent nucleotides with a different sequence no more than six nucleotides long. Complex mutations type II are defined as replacements where the original sequence or the new sequence or both are longer than six nucleotides.

^fOther mutations for the *pol3*-Y708A and *pol3*-Y708A *rev3* Δ strains are described in Ref. 22. Other mutations in the *pol3*-Y708A *rev1*-1 and *pol3*-Y708A *rev1*-cd strains were a six-nucleotide insertion and a three-nucleotide insertion, respectively.

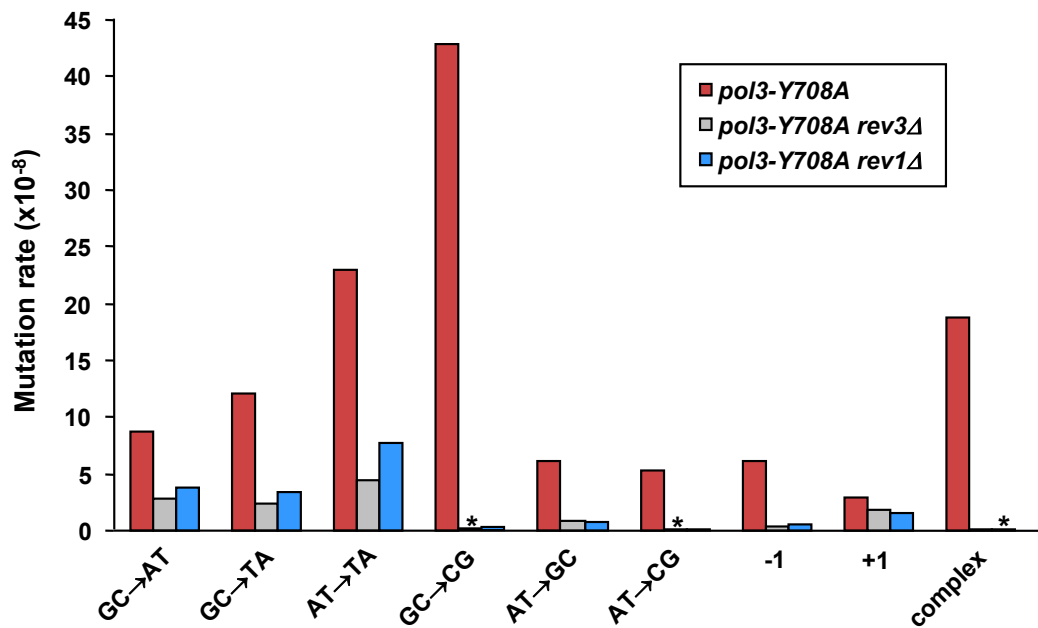


Fig. S1. The presence of Rev1 is required for all Pol-dependent DRIM events. The bars show rates for individual types of *can1* mutations in the *pol3-Y708A* strain and its *rev3* and *rev1* derivatives. Data are from Table S2. The asterisks indicate maximal mutation rate in cases where no mutations of the particular type were detected.

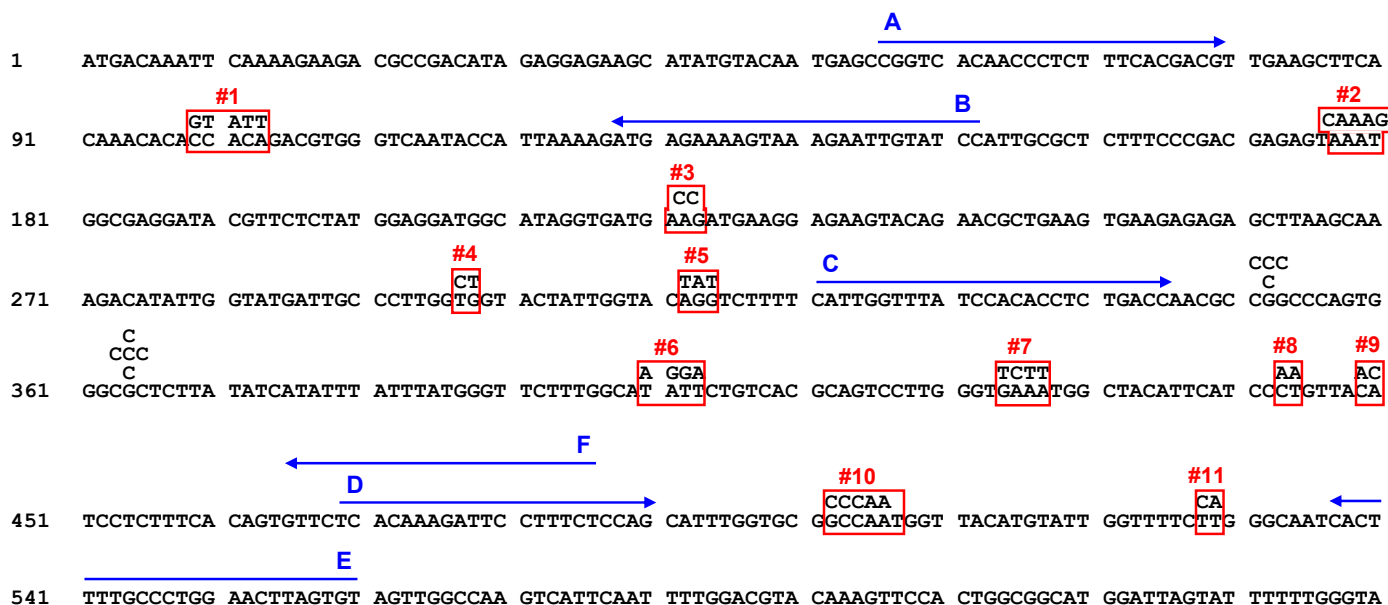


Fig. S2. Oligonucleotide primers for *in vitro* studies of DNA polymerase pausing during copying of the *CAN1* sequence. The first 629 bases of the *CAN1* coding sequence are shown. Complex mutations and G→C base substitution hotspots observed in the *pol3-Y708A* strain (22) are shown by red boxes and letters above the sequence, respectively. Blue arrows indicate the annealing sites for the oligonucleotides used in the experiments shown in Figures 2, 3, 5 and 8.

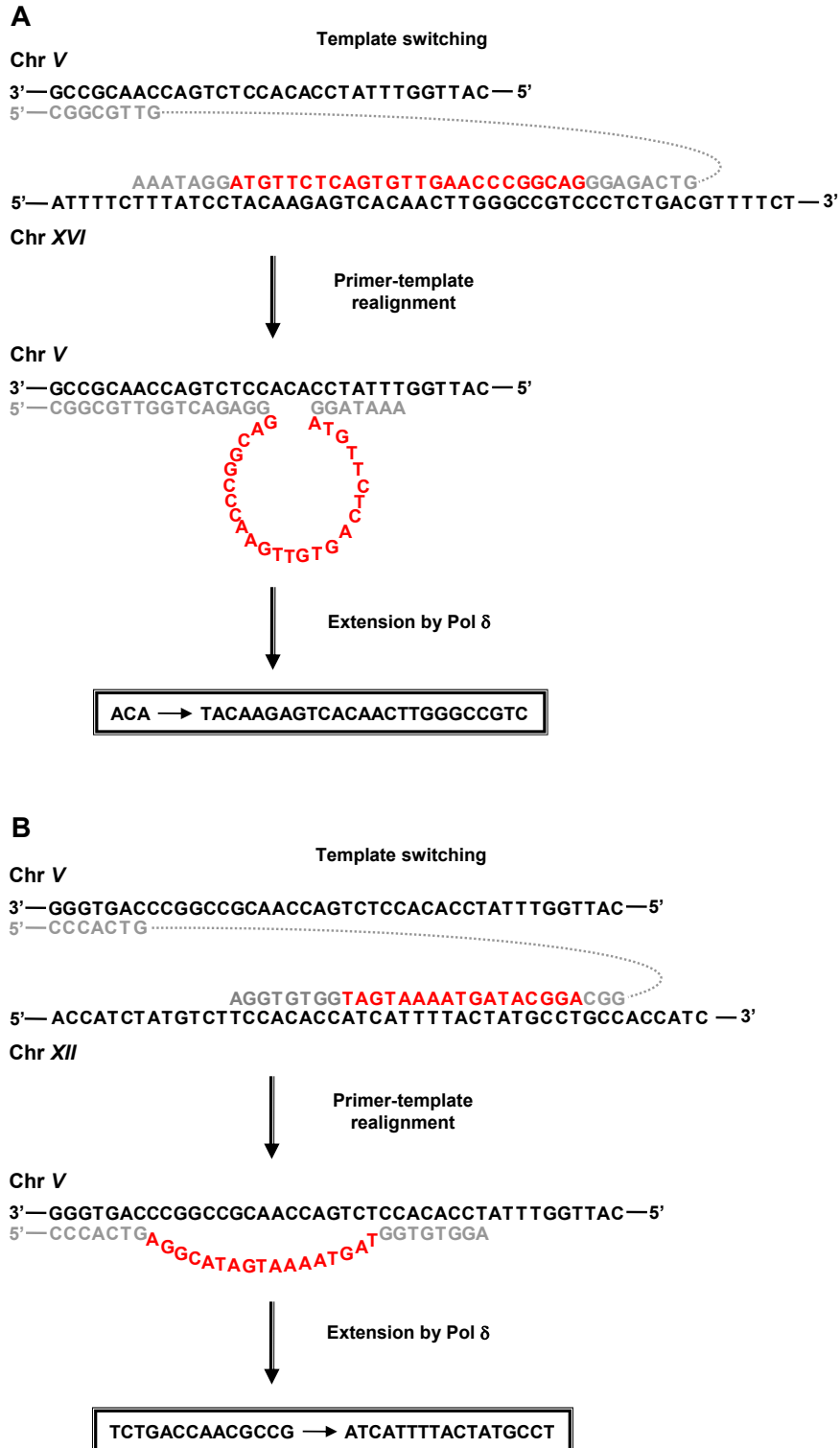
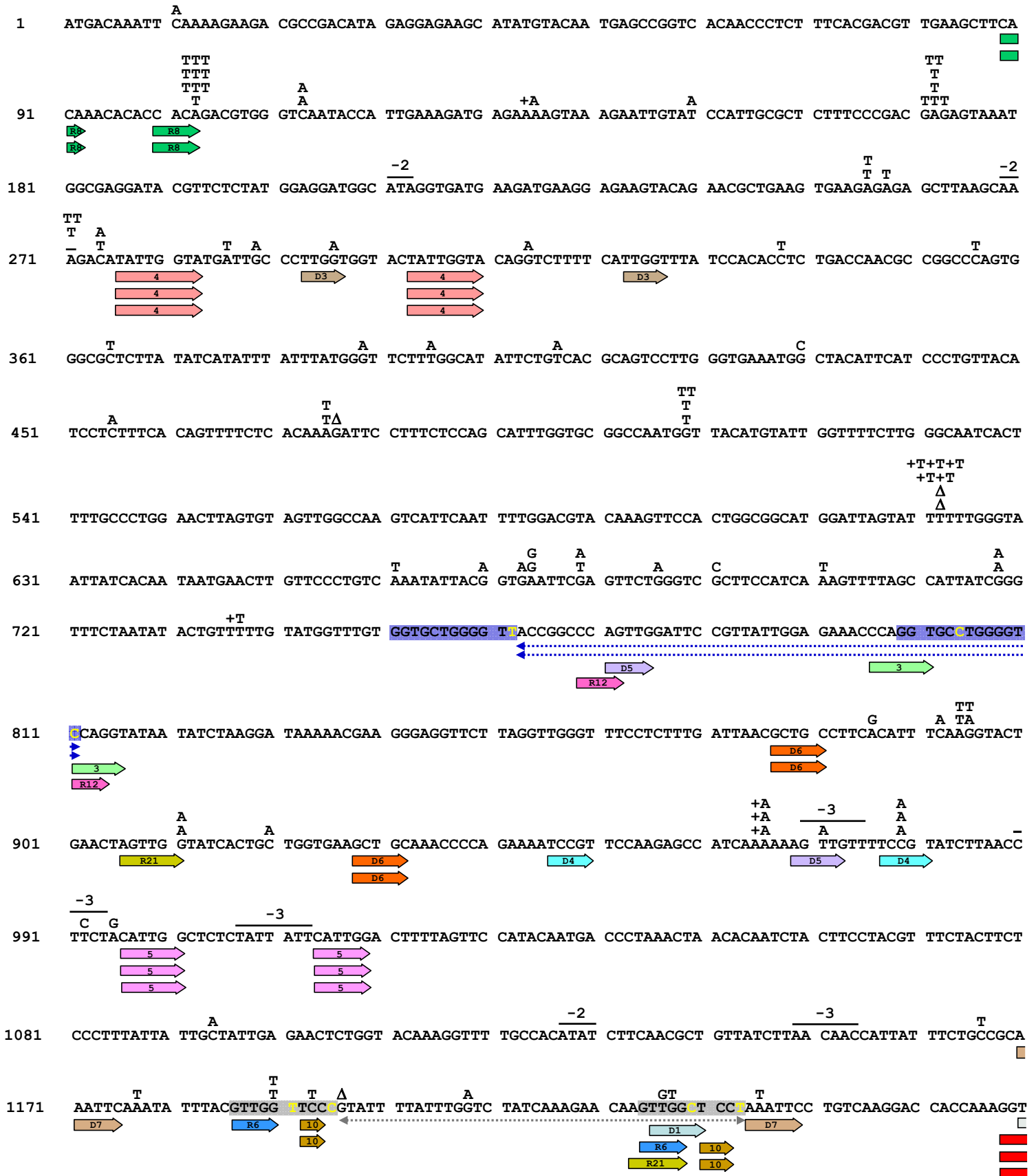


Fig. S4. Trans-chromosomal template-switching events occur in the absence of Pol. Panels A and B show models that explain the formation of two type II complex mutations observed in the *pol3-Y708A rev3* strain (Ref. 22; Fig. S3).

Fig. S5. Spectra of spontaneous *can1* mutations in the *pol3-Y708A rev1Δ* (A), *pol3-Y708A rev1-cd* (B), *mms2Δ* (C), *mms2Δ pol3-Y708A* (D) and *mms2Δ pol3-Y708A rev1-cd* (E) strains. The coding sequence of the *CAN1* gene is shown. Letters and triangles above the sequence indicate base substitutions and single base deletions, respectively. Letters with a “+” symbol indicate single base insertions. Red boxes show complex mutations. Deletions of two to five nucleotides are designated by a line above the sequence with a number of deleted nucleotides above the line. For larger deletions, short direct repeats flanking the deleted region are shown by colored arrows with a deletion identification number inside the arrow. Dashed colored lines below the *CAN1* sequence show multi-base deletions between imperfect direct repeats. The corresponding imperfect repeats are indicated by the same-color shaded boxes. The non-identical nucleotides within the repeats are in yellow. A deleted region that is not flanked by short repeats is shown by a green dashed line below the *CAN1* sequence. A 38-nucleotide duplication is indicated by a black line above the sequence. The green box shows a three-nucleotide insertion. A complex mutation that was observed twice has a red 2X above the box.

A *pol3-Y708A rev1Δ*

B *pol3-Y708A rev1-cd*

-15 AAAAA ⁱAGGCATAGCA

1 ATGACAAATT CAAAAGAAGA CGCCGACATA GAGGAGAAGC ATATGTA^AGGCAA TGAGCCGGTC ACAACCCCTCT TTCACGACGT TGAAGCTTCA

91 CAAACACACC ACAGACGTGG GTCAATACCA TTGAAAGATG AGAAAAGTAA AGAATTTGTAT CCATTGCGT^Δ CTTTCCCAGC GAGAGTAAAT

181 ATACAATTCTT GGCGAGGATA CGTTCTCTAT GGAGGATGGC ATAGGTGATG duplication AAGATGAAGG AGAAGTACAG AACGCTGAAG TGAAGAGAGA AT G GCTTAAGCAA

271 AGACATATTG GTATGATTGC CCTTGGTGGT ACTATTGGTA ACA^TCA^TA^A CAGGTC^TTTT CATTGGTTTA TCCACACCTC TGACCAACGC CGGCCCGATG ^{A4}→

361 GGCGCTCTTA TATCATATTT ATTTATGGGT TCTTTGGCAT ATTCTGTAC GCAGTCTTG GGTGAAATGG CTACATTCAT CCCTGTTACA

451 TCCTCTTTCA CAGTTTTCTC ACAAAGATTC CTTTCTCCAG CATTGGTGC GGCCAATGGT TACATGTATT CTAAG GGTTTTCTTG GGCAATCACT

541 TTTGCCCTGG AACTTAGTGT GTTGCCAA GTCATTCAAT TTTGGACGTA CAAAGTTCCA CTGGCGGCAT GGATTAGTAT TTTTGGGTA

631 ATTATCACAA TAATGAACTT GTTCCCTGTC AAATATTACG GTGAATCGA GTTCTGGGTC GCTTCCATCA AAGTTTTAGC CATTATCGGG

721 TTTCTAATAT ACTGTTTTTG TATGGTTTGT GGTGCTGGGG TTACCGGCC AGTTGGATTC CGTTATTGGA GAAACCCAGG TGCCTGGGGT

811 CCAGGTATAA TATCTAAGGA TAAAAAGAA GGGAGTTCT TAGGTTGGGT TTCCTCTTTG ATTAACGCTG CCTTCACATT TCAAGGTACT

901 GAACTAGTTG GTATCACTGC TGGTGAAGCT GCAAACCA GAAAATCCGT TCCAAGAGCC ATCAAAAAAG AATTTTTTTTGC TATCTTAACC

991 TTCTACATTG GCTCTCTATT ATTCATTGGA CTTTtagTTC CATACAATGA CCCTAAACTA ACACAATCTA CTTCTACGT TTCTACTTCT

1081 CCCTTTATTA TTGCTATTGA GAACTCTGGT ACAAAGGTTT TGCCACATAT CTTCAACGCT GTTATCTTAA CAACCATTAT TTCTGCCGCA

1171 AATTCAAATA TTTACGTTGG TTCCCGTATT TTATTTGGTC TATCAAAGAA CAAGTTGGCT CCTAAATTCC TGTC AAGGAC CACCAAAGGT

1261 GGTGTTCCAT ACATTGCAGT TTTTCGTACT GCTGCTTCATTG GCGCTTTGGC TTACATGGAG ACATCTACTG GTGGTGACAA AGTTTTCGAA

1351 TGCTATTAA ATATCACTGG TGTTGCAGGC TTTTTTGCAT GGTATTTAT CTCAATCTCG CACATCAGAT TTATGCAAGC TTTGAAATAC

1441 CGTGGCATCT CTCGTGACGA GTTACCATT TAAAGCTAAAT TAATGCCCGG CTGGCTTAT TATGGGCCA CATTATGAC GATCATTATC

1531 ATTATTCAAG GTTTCACGGC TTTTGCACCA AAATTC AATG GTGTTAGCTT TGCTGCCGCC TATACTCTA TTTTCTGTT CTTAGCTGTT

1621 TGGATCTTAT TTCAATGCAT ATTCAGATGC AGATTTATTT GGAAGATTGG AGATGTCGAC ATCGATTCCG ATAGAAGAGA CATTGAGGCA

1711 ATTGTATGGG AAGATCATGA ACCAAAGACT TTTTGGGACA AATTTGGAA TGTTGTAGCA TAG

C *mms2Δ*

1 ATGACAAATT^Δ CAAAAGAAGA^G CGCCGACATA^T GAGGAGAAGC^A ATATGTACAA^A TGAGCCGGTC ACAACCCCTCT TTCACGACGT TGAAGCTTCA
 91 CAAACACACC^T ACAGACGTGG^A GTCAATACCA^Δ TTGAAAGATG^G AGAAAAGTAA^T AGAATTGTAT^T CCATTGCGCT CTTTCCCGAC^Δ GAGAGTAAAT
 181 GGCGAGGATA^T CGTTCTCTAT^Δ GGAGGATGGC^A ATAGGTGATG^T AAGATGAAGG^T AGAAGTACAG^T AACGCTGAAG^{AA} TGAAGAGAGA^C GCTTAAGCAA^Δ
 271 AGACATATTG^G GTATGATTGC^C CCTTGGTGGT^A ACTATTGGTA^{AC} CAGGTCTTTT^{AT} CATTGGTTTA^{CT} TCCACACCTC^A TGACCAACGC^A CGGCCAGTG^Δ
 361 GGCGCTCTTA^G TATCATATTT^G ATTTATGGGT^A TCTTTGGCAT^C ATTCTGTAC^C GCAGTCCTTG^C GGTGAAATGG^G CTACATTCAT^G CCTGTTACA^{TT}
 451 TCCTCTTTCA^T CAGTTTTCTC^G ACAAAGATTC^Δ CTTTCTCCAG^G CATTGGGTGC^A GGCCAATGGT^Δ TACATGTATT^A GGTTTTCTTG^C GGCAATCACT^C
 541 TTTGCCCTGG^T AACTTAGTGT^G GTTGGCCAA^Δ GTCATTCAAT^A TTTGACGTA^A CAAAGTTCCA^Δ CTGGCGGCAT^A GGATTAGTAT^{AA} TTTTGGGTA^Δ
 631 ATTATCA^{AAA} CAA^A TAATGA^T AACTT^A GTTCCCTGTC^A AAATATTACG^T GTGAATTCGA^C GTTCTGGGTC^T GCTTCCA^A CCATTTTGG^Δ AAGTTT^Δ TAGC^Δ CATTATCGGG
 721 TTTCTAATAT^A ACTGTTTTTG^T TATGGTTTGT^A GGTGCTGGGG^T TTACCGCCCC^Δ AGTTGGATTC^Δ CGTTATTGGA^C GAAACCCAGG^A TGCTGGGGT^Δ
 811 CCAGGTATAA^C TATCTAAGGA^A TAAAAACGAA^Δ GGGAGGTCT^A TAGGTTGGGT^A TTCCTCTTTG^T ATTAACGCTG^T CCTTCACATT^A TCAAGGTACT^T
 901 GAACTAGTTG^C GTATCACTGC^A CTAAC^T A^A TGGTGAAGCT^G GCAAACCCCA^Δ GAAAATCCGT^T TCCAAGAGCC^C ATCAA^Δ AAG^Δ TTTTCCG^T TATCTTAACC^{TA}
 991 TTCTACATTG^{CC} GCTCTCTATT^C ATTCATTGGA^A CTTTTAGTTC^Δ CATACAATGA^T CCCTAAACTA^T ACACAATCTA^{+A} CTTCTACGT^Δ TTCTACTTCT^A
 1081 CCCTTTATTA^C TTGCTATTGA^A GAACTCTGGT^T ACAAAAGTTT^C TGCCACATAT^{M1} CTTCACGCT^Δ GTTATCTTAA^G CAACCATTAT^G TTCTGCCGCA^G
 1171 AATTCAAATA^A TTTTACGTTGG^T TTCCCCTATT^{AG} TTATTTGGTC^{TT} TATCAA^G GAA^T CAAGTTGGCT^{CT} CCTAAATTC^G TGTC AAGGAC^{GG} CACCAAAGGT^A
 1261 GGTGTTCCAT^{CT} ACATTGCAGT^A TTTTCGTTACT^C GCTGCATTTG^A GCGCTTTGGC^Δ TTACATGGAG^Δ ACATCTACTG^G GTGGTGACAA^Δ AGTTTTCGAA^Δ
 1351 TGGCTATTAA^T ATATCACTGG^T TGTTGCAGGC^T TTTTTTGCAT^{AA} GGTTATTTAT^G CTCAATCTCG^G CACATCAGAT^T TTATGCAAGC^T TTTGAAATAC^T
 1441 CGTGGCATCT^A CTCGTGACGA^Δ GTTACCATT^A AAAGCTAAAT^A TAATGCCC^Δ CGTGGCTTAT^{+T} TATGCGGCCA^Δ CATTATGAC^Δ GATCATTATC^T
 1531 ATTATTCAAG^A GTTTCACGGC^Δ TTTTGCACCA^Δ AAATTC AATG^Δ GTGTAGCTT^{AA} TGCTGCCGCC^Δ TATATCTCTA⁻² TTTTCTGT^A CTTAGCTGTT^A
 1621 TGGATCTTAT^A TTCAATGCAT^Δ ATTCAGATGC^Δ AGATTTATTT^Δ GGAAGATTGG^Δ AGATGTCGAC^Δ ATCGATTCCG^Δ ATAGAAGAGA^Δ CATTGAGGCA^Δ
 1711 ATTGTATGGG^A AAGATCATGA^Δ ACCAAAGACT^Δ TTTTGGGACA^Δ AATTTTGGAA^Δ TGTGTAGCA^Δ TAG^Δ

D *mms2Δ pol3-Y708A*

1 ATGACAAATT^Δ CAAAAGAAGA^G CGCCGACATA GAGGAGAAGC^G ATATGTACAA^G TGAGCCGGTC^Δ ACAACCCCTCT TTCACGACGT TGAAGCTTCA^A
 91 CAAACACACC^T ACAGACGTGG^{GG} GTCAATACCA^A TTGAAAGATG^{G T} AGAAAAGTAA AGAATTGTAT CCATTGCGCT CTTTCCCAGC GAGAGTAAAT^G
 181 GGCGAGGATA CGTTCTCTAT^Δ GGAGGATGGC ATAGGTGATG AAGATGAAGG AGAAGTACAG AACCTGGAAG^Δ TGAAGAGAGA GCTTAAGCAA^G
 271 AGACATATTG^G GTATGATTGC^{A C} CTTTGGTGGT^{CTG} ACTATTGGTA^G CAGGTCTTTT^C CATTGGTTTA^C TCCACACCTC TGACCAACGC^C CGGCCAGTG^C
 361 GGCGCTCTTA^C TATCATATTT^G ATTTATGGGT⁻⁴ TCTTTGGCAT^Δ ATTCTGTCAC^G GCAGTCCTTG^{TC A A} GGTGAAATGG^C CTACATTCAT^A CCCTGTTACA^A
 451 TCCTCTTTCA CAGTTTTCTC^T ACAAAGATTC^Δ CTTTCTCCAG CATTGGTGC GGCCAATGGT TACATG^{GGCC}TATT^Δ GGTTTTCTTG GCAATCACT^A
 541 TTTGCCCTGG AACTTAGTGT AGTTGGCCAA GTCATTCAAT TTTGGACGTA CAAAGTTCCA CTGGCGGCAT GGATTAGTAT^C TTTTGGGTA^A
 631 ATTATCACAA^G TAATGAACTT^{CC} GTTCCCTGTC^{AA} AAATATTACG^A GTGAATTCGA^T GTTCTGGGTC^{C G} GCTTCCATCA^{CT} AAGTTTTAGC^G CATTATCGGG^C
 721 TTTCTAATAT^{TG} ACT^ATTTTTG^A TATGGTTTGT^{CGTTTTTT} GGTGCTGGGG^Δ TTACCGGCC AGTTGGATTC CGTTATTGGA GAAACCCAGG^C TGCCTGGGGT^Δ
 811 CCAGGT^{AAAAA}TATAA^Δ TATCTAAGGA^T TAAAAACGAA GGGAGTTCT TAGGTTGGGT TTCCTCTTTG^C ATTAACGCTG^A CCTTCACATT^A TCAAGGTACT^{GG}
 901 GAACTAGTTG GTATCACTGC^{A T CG} TGGTGAAGCT^T GCAAACCCCA^{AA} GAAAATCCGT^{T A} TCCAAG^{ACT}GCC^{+A} ATCAA^{CT}AAAG^{ATGG} TTGTTTTCGG^{C AT} TATCTTAACC^G
 991 TTCTACATTG^G GCTCTCTATT^T ATTCATTGGA^{T G} CTTT^TTAGTTC^G CATACAATGA^G CCCTAAACTA^G ACACAATCTA^G CTTCTACGT^G TTCTACTTCT^G
 1081 CCCTTTATTA^A TTGCTATTGA^{TΔ} GAACTCTGGT ACAAAGGTTT TGCCACATAT CTTCAACGCT GTTATCTTAA^G CAACCATTAT^G TTCTGCCGA^G
 1171 AATTCAAATA^G TTTACGTTGG^{GG} TTCCCCTAT^TT^A TTATTT^GGGTC^G TATCAAAGAA^G CAAGTTGGCT^A CCTAAATTC^{GG} TGTCAAGGAC^G CACCAAAGGT^Δ
 1261 GGTTGTTCCAT^T ACATTGCAGT^G TTTCGTTACT^A GCTGCATTG^A GCGCTTTGGC^A TTACATGGAG^G ACATCTACTG^{GG} GTGGTGACAA^G AGTTTTCGAA^Δ
 1351 TGGCTATTAA^{AAA T} ATATCACTGG^A TGTTGCAGGC^{CG T} TTTTTGTCAT^T GGTATTAT^G CTCAATCTCG^{GG} CACATCAGAT^{A C T} TTATGCAAGC^Δ TTTGAAATAC^Δ
 1441 CGTGGCATCT^Δ CTCGTGACGA GTTACCATT^T AAAGCTAAAT^{Δ G} TAATGCCCGG^{TT} CTGGCTTAT^G TATGCGCCA CATTATGAC^G GATCATTATC^G
 1531 ATTATTCAAG^T GTTTCACGGC TTTTGCACCA AAATTCAATG GTGTTAGCTT TGCTGCCGCC TATATCTCTA TTTTCTGTG^A CTTAGCTGTT^A
 1621 TGGATCTTAT^A TTCAATGCAT^T ATTCAGATGC^{2x} AGATTTATTT^{TG} GGAAGATTGG^A AGATGTCGAC^Δ ATCGATTCCG^Δ ATAGAAGAGA CATTGAGGCA^Δ
 1711 ATTGTATGGG AAGATCATGA ACCAAAGACT TTTTGGGACA AATTTTGGAA TGTGTAGCA TAG

E *mms2Δ pol13-Y708A rev1-cd*

