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)	-
rev1Δ	25 (20-30)	1.6
rev1-1	14 (12-16)	0.9
rev1-cd	19 (14-28)	1.3
pol3-Y708A	140 (130-160)	9.3
rev1Δ pol3-Y708A	26 (24-29)	1.7
rev1-1 pol3-Y708A	47 (39-52)	3.1
rev1-cd pol3-Y708A	79 (68-97)	5.3
mms2Δ	87 (74-120)	5.8
mms2Δ rev1-cd	110 (91-130)	7.3
mms2Δ pol3-Y708A	250 (220-290)	17
mms2Δ rev1-cd pol3-Y708A	240 (200-300)	16

[a]Mutation 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	*pol3-Y708A*		*pol3-Y708A rev3Δ*		*pol3-Y708A rev1Δ*		*pol3-Y708A rev1-1*		*pol3-Y708A rev1-cd*		*mms2Δ*		*mms2 pol3-Y708A*		*mms2Δ pol3-Y708A rev1-cd*	
	No.[a]	MR[c] ($\times10^{-8}$)	No.[a]	MR[d] ($\times10^{-8}$)	No.[b]	MR[d] ($\times10^{-8}$)	No.	MR[d] ($\times10^{-8}$)	No.[b]	MR[d] ($\times10^{-8}$)	No.[b]	MR[d] ($\times10^{-8}$)	No.[b]	MR[d] ($\times10^{-8}$)	No.[b]	MR[d] ($\times10^{-8}$)
Base substitutions																
GC → AT	11	8.6	23	2.9	30	3.9	25	6.3	28	12	49	20	28	38	54	62
AT → 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 → TA	16	12	20	2.5	27	3.5	22	5.5	40	17	43	17	31	42	50	58
GC → CG	55	43	0	<0.13	2	0.26	21	5.3	37	15	41	16	53	71	29	33
AT → 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 → 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

[a]Data for the *pol3-Y708A* and *pol3-Y708A rev3Δ* strains are from Ref. 22.
[b]Full mutational spectra are shown in Fig. S5.
[c]Two *can1* mutants of the *pol3-Y708A* strain, one of the *pol3-Y708A rev1Δ* strain, one of the *pol3-Y708A rev1-1* strain and four of the *mms2Δ* 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.
[d]Rate for each type of mutation was calculated as follows: $MR_i=(M_i/M_T)xMR$, 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.
[e]Complex 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.
[f]Other mutations for the *pol3-Y708A* and *pol3-Y708A rev3Δ* 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.

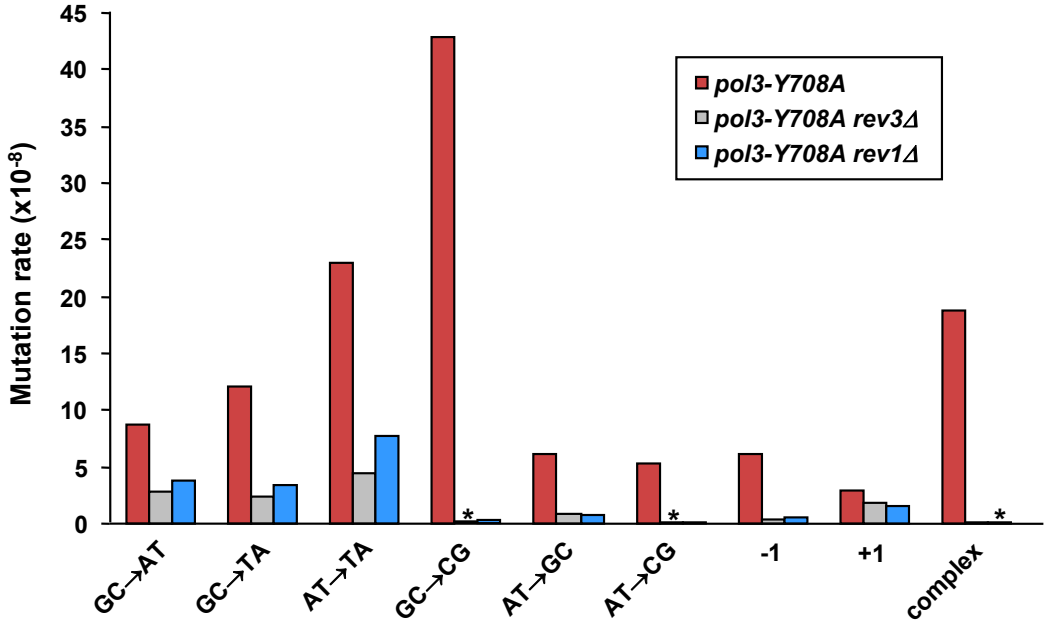


Fig. S1. The presence of Rev1 is required for all Pol-dependent DRIM events. The bars show rates for individual types of *can1* mutations in the *pol3-Y708A* strain and its *rev3* and *rev1* derivatives. Data are from Table S2. The asterisks indicate maximal mutation rate in cases where no mutations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

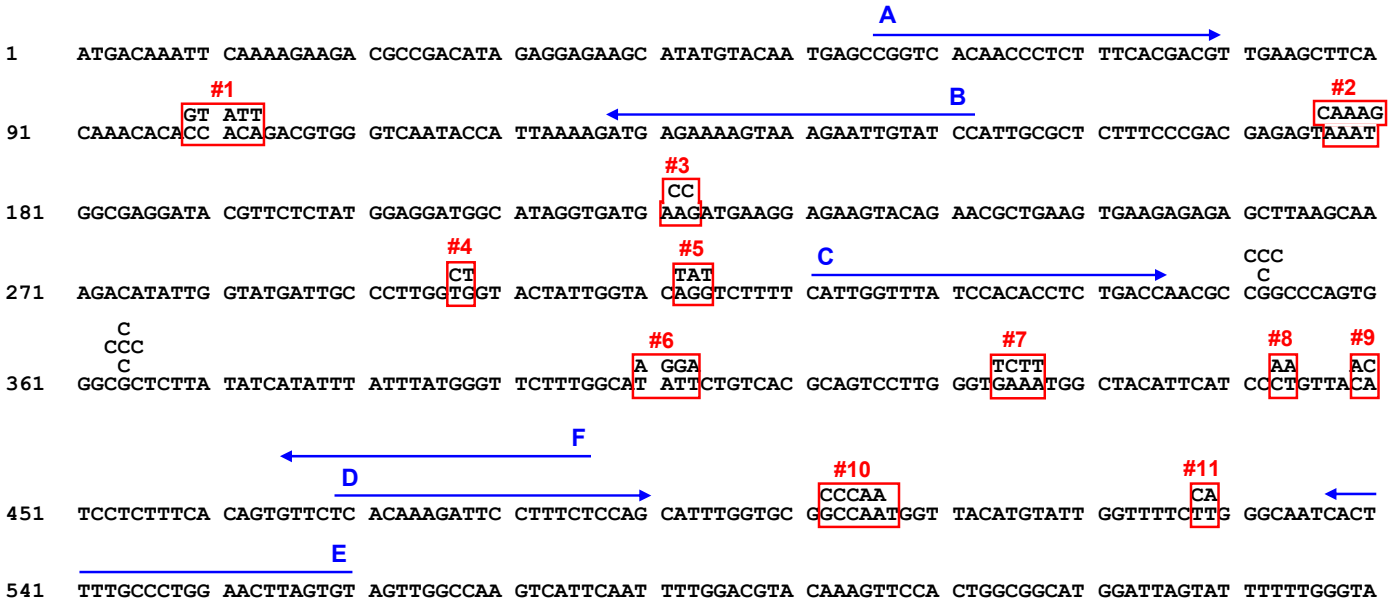


Fig. S2. Oligonucleotide primers for *in vitro* studies of DNA polymerase pausing during copying of the *CAN1* sequence. The first 629 bases of the *CAN1* coding sequence are shown. Complex mutations and G→C base substitution hotspots observed in the *pol3-Y708A* strain (22) are shown by red boxes and 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.

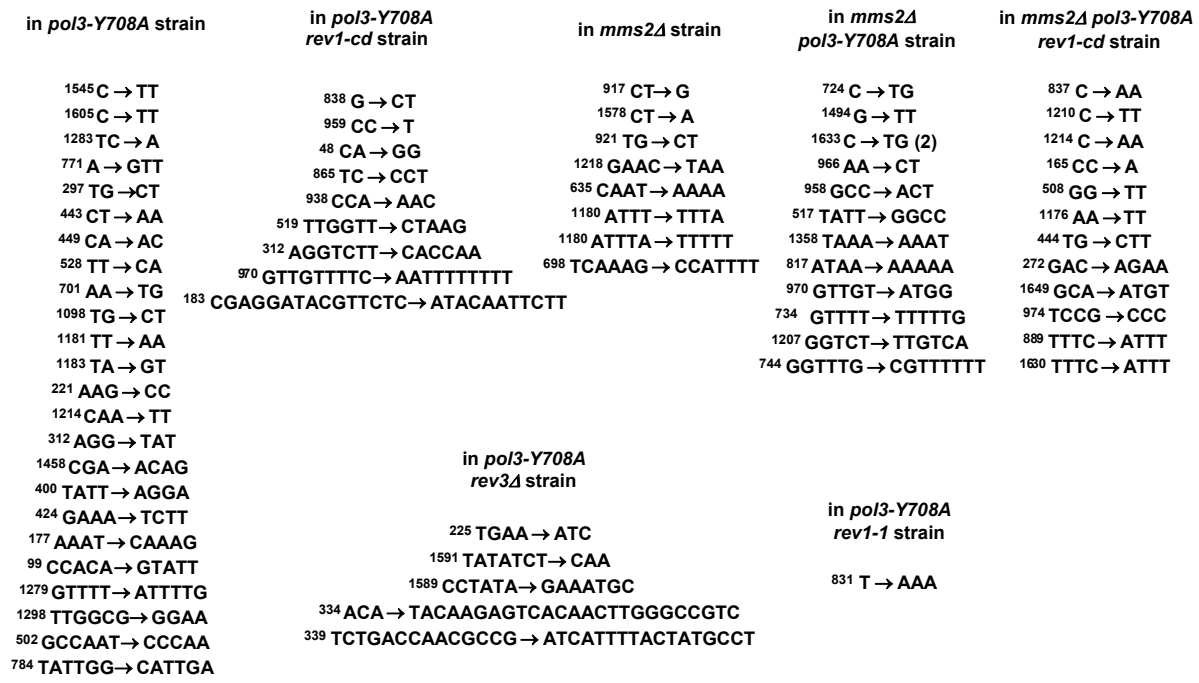


Fig. S3. Complex mutations found in the *CAN1* gene of the replication and DNA damage tolerance mutants. The superscript numbers indicate nucleotide position. Data for the *pol3-Y708A* and *pol3-Y708A rev3* strains are from Ref. 22

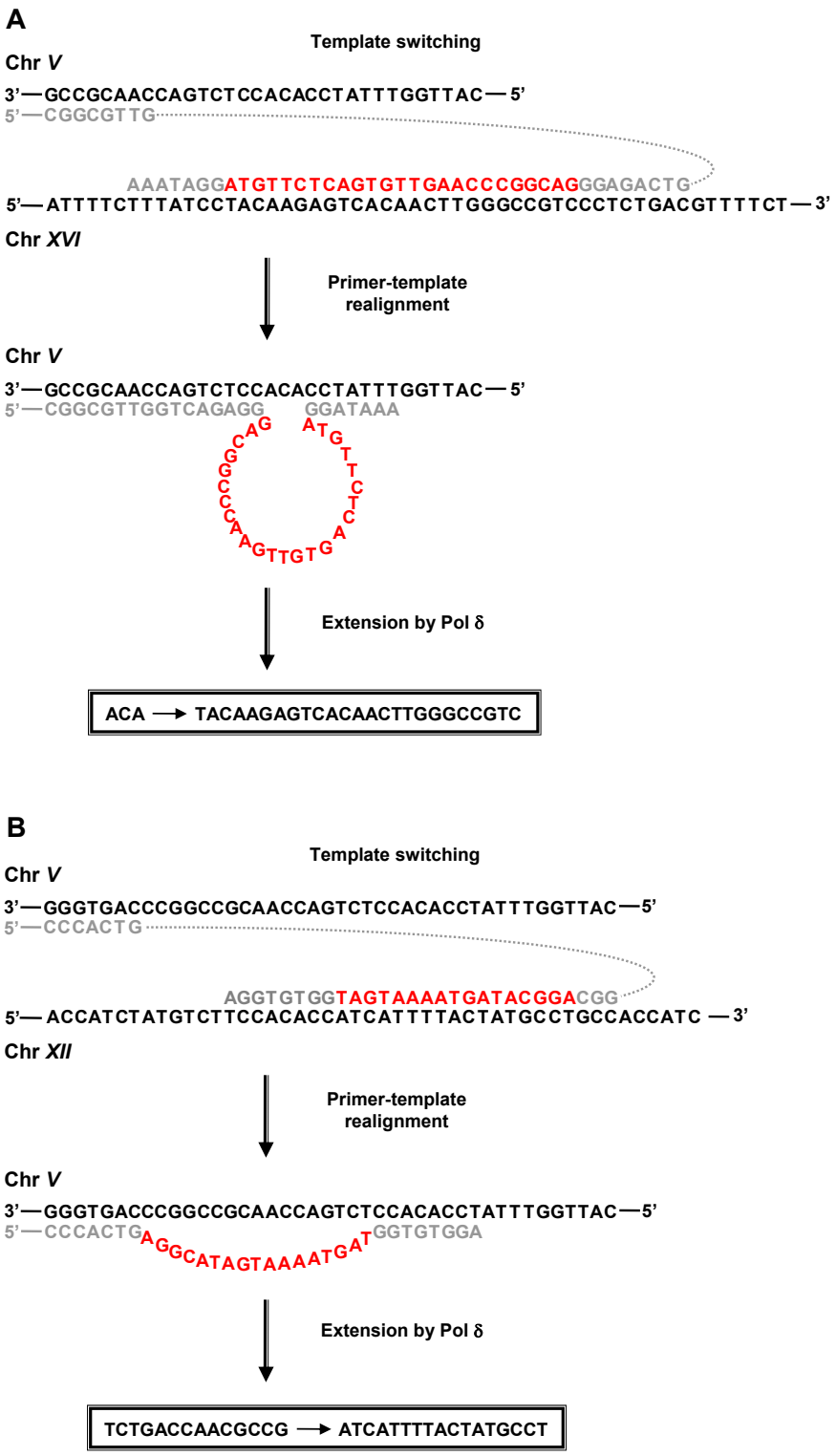
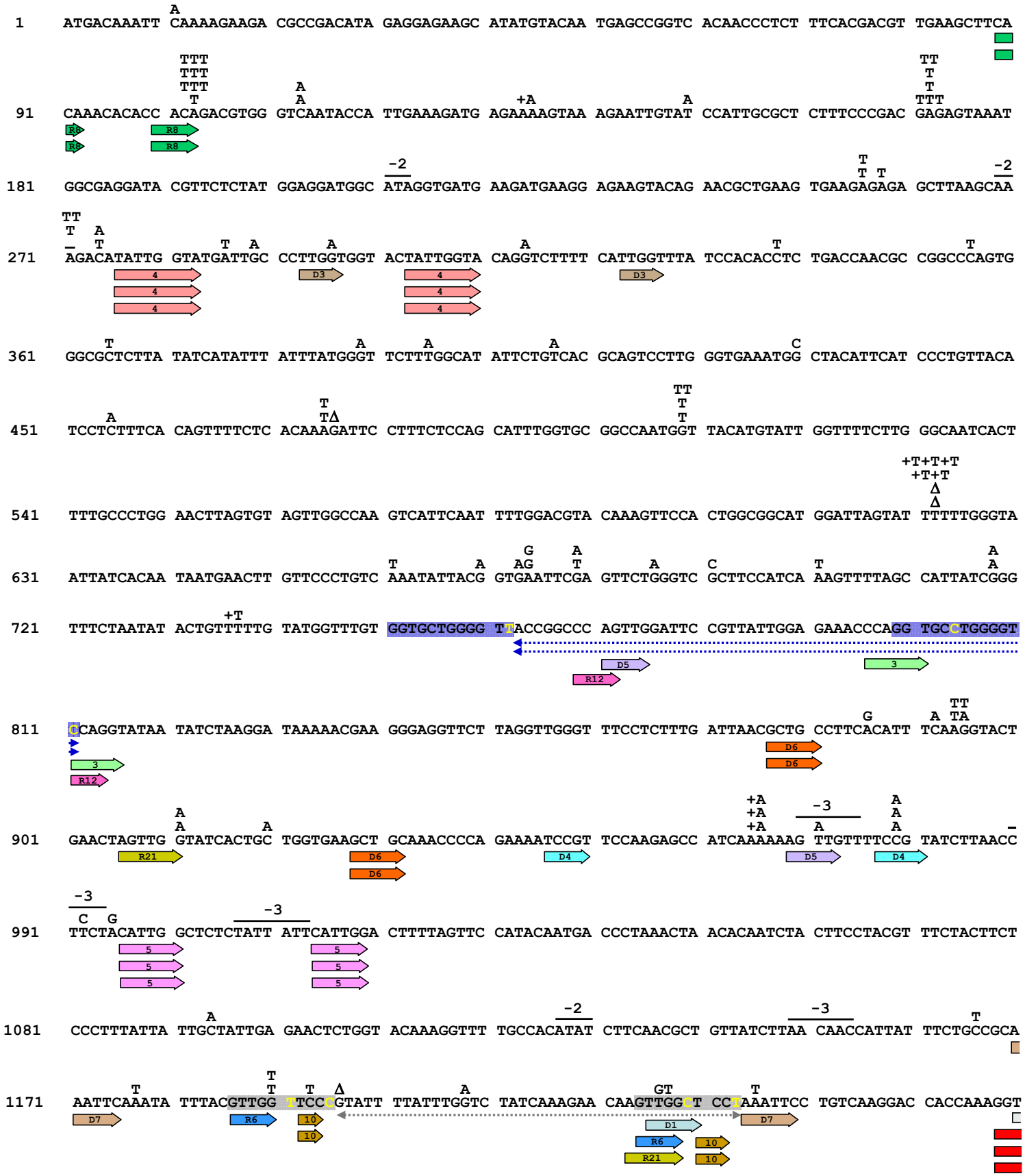


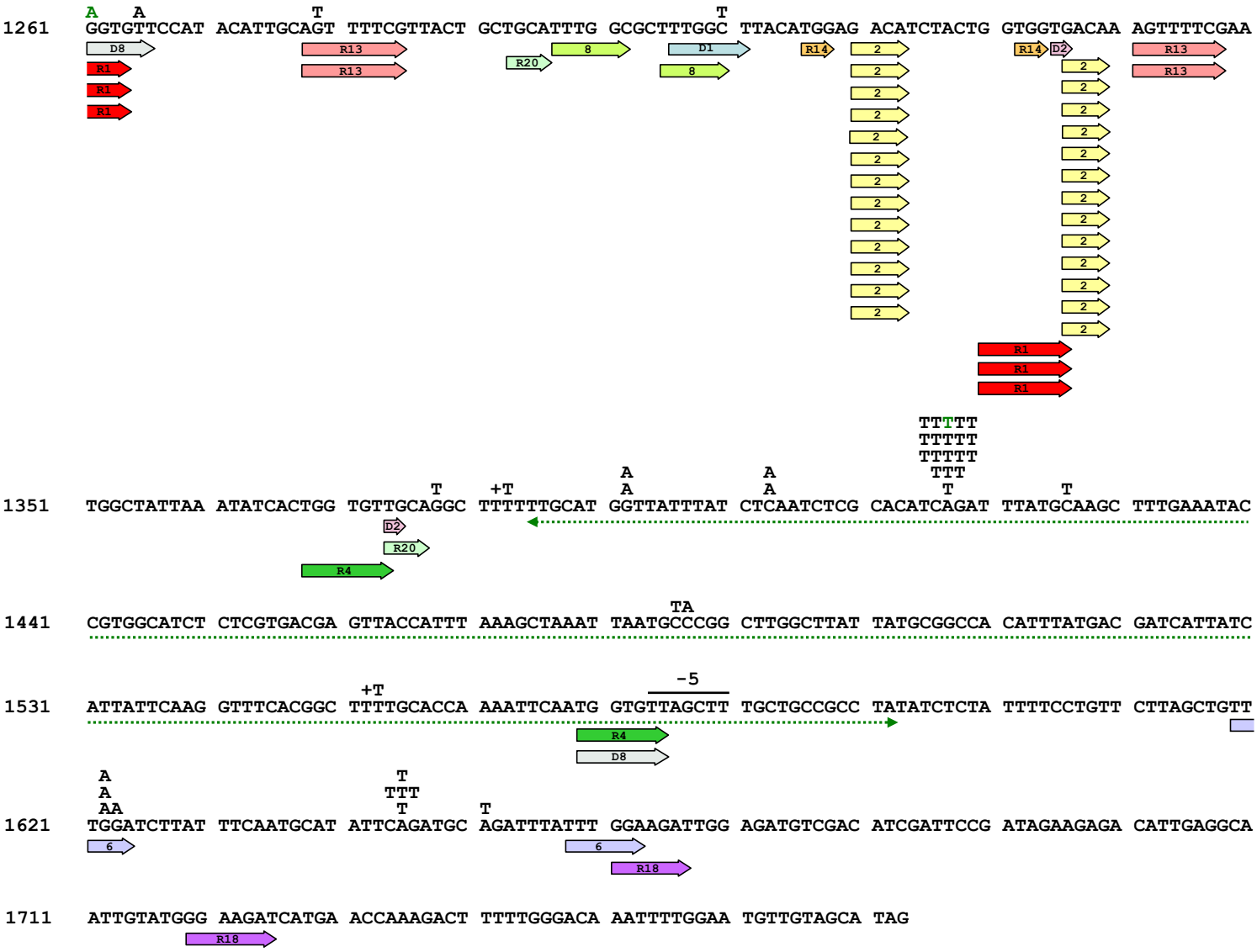
Fig. S4. Trans-chromosomal template-switching events occur in the absence of Pol. Panels A and B show models that explain the formation of two type II complex mutations observed in the *pol3-Y708A rev3* strain (Ref. 22; Fig. S3).

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.

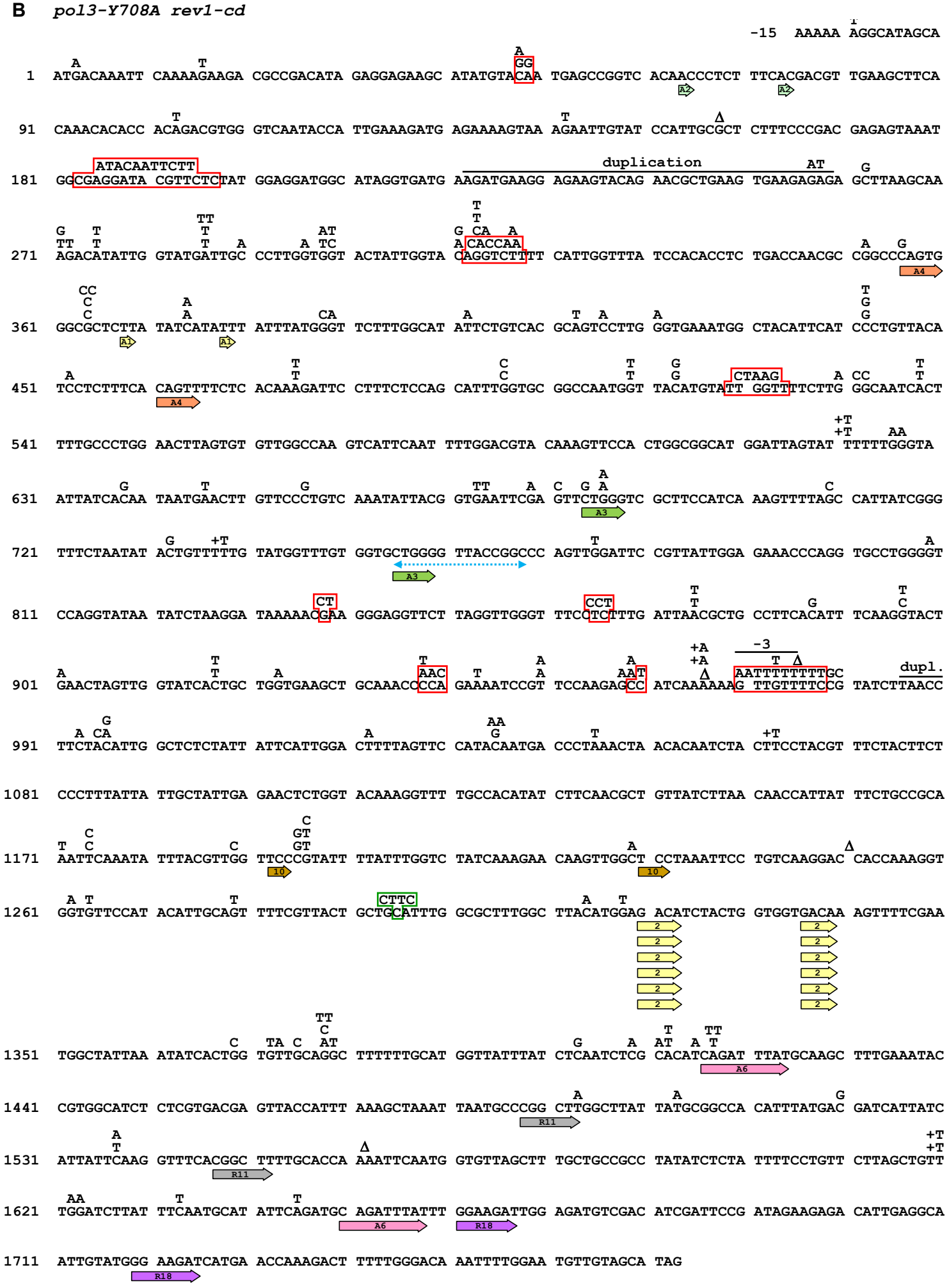
A *pol3-Y708A rev1Δ*



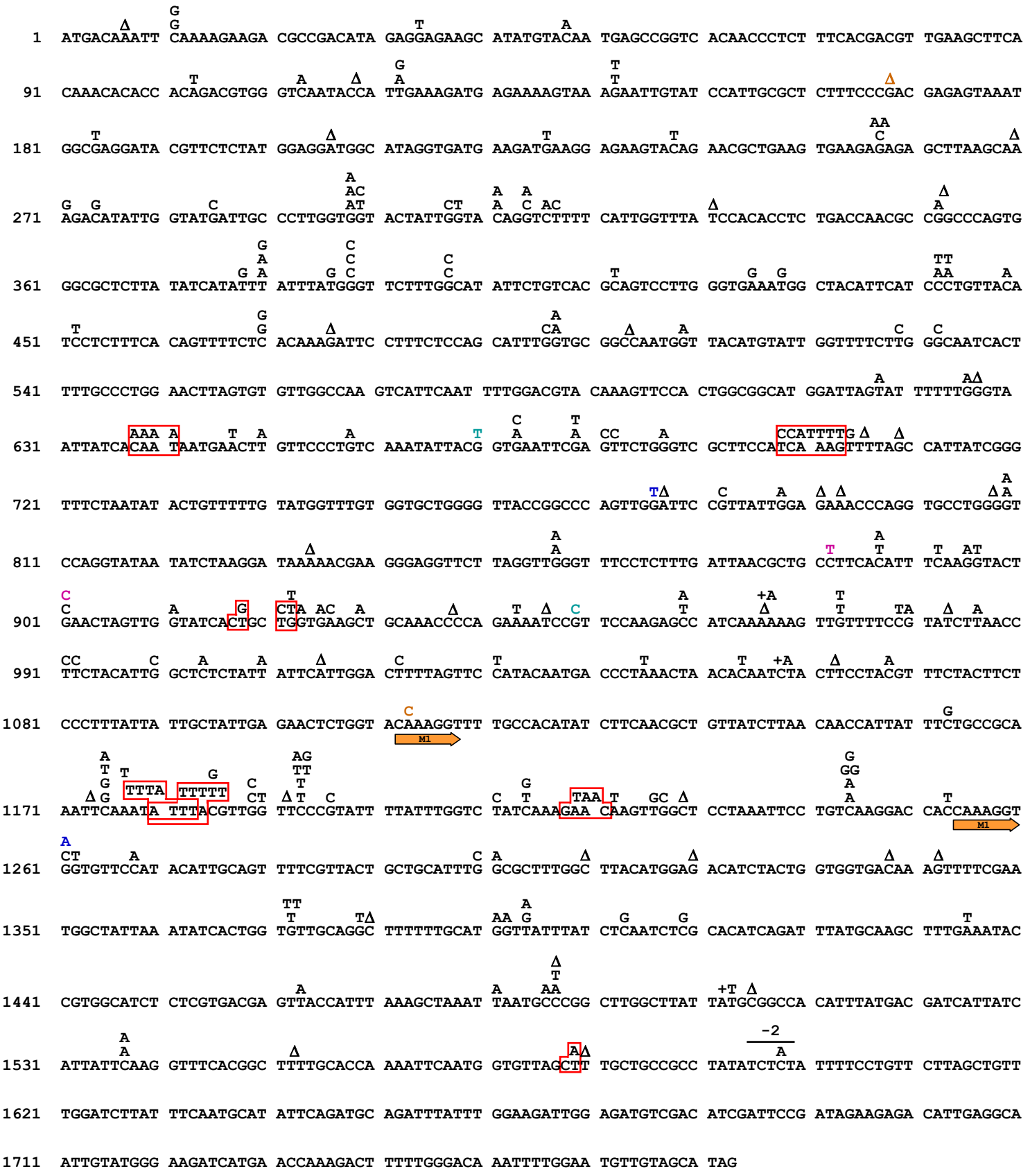
***pol3-Y708A rev1Δ* cont.**



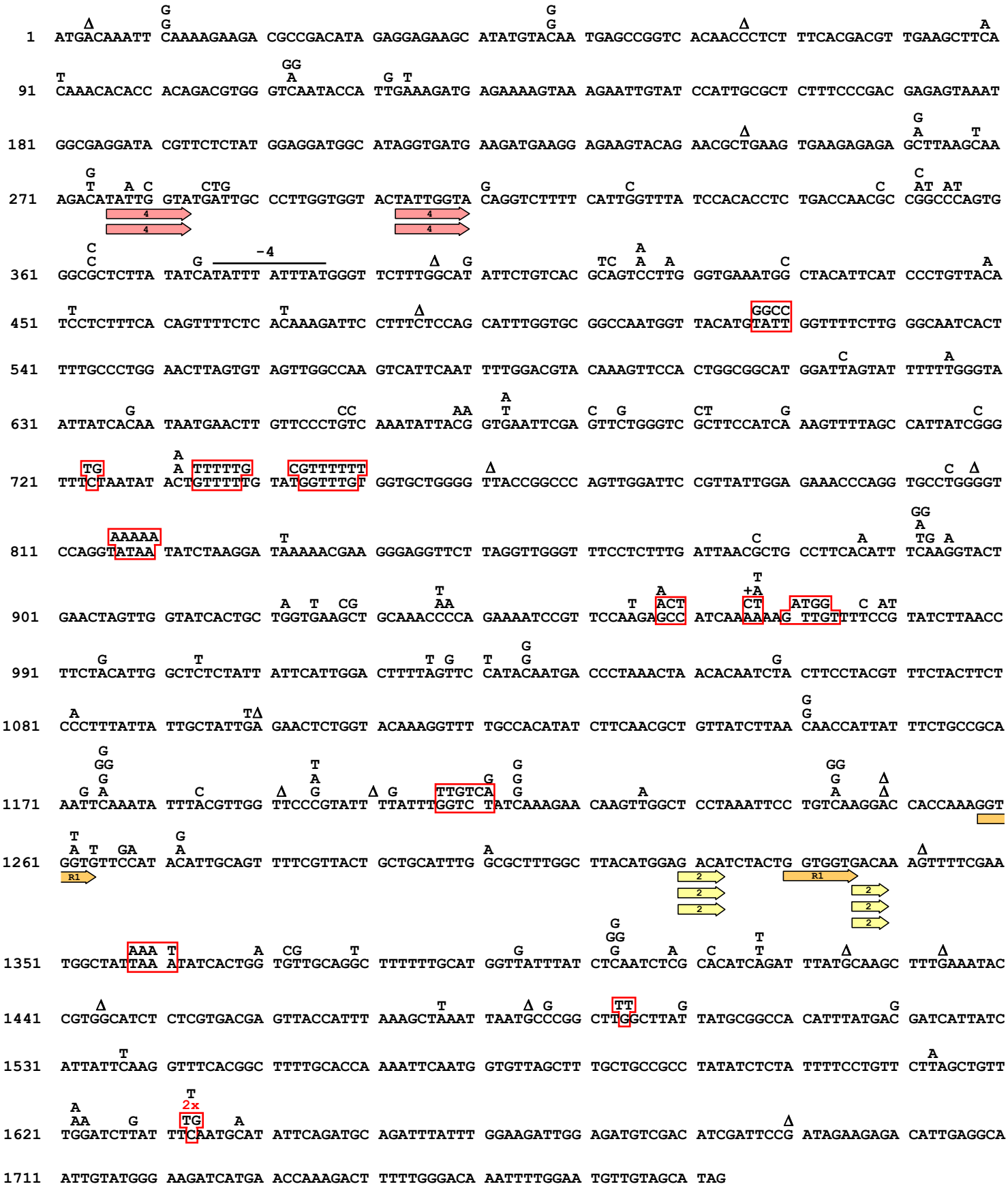
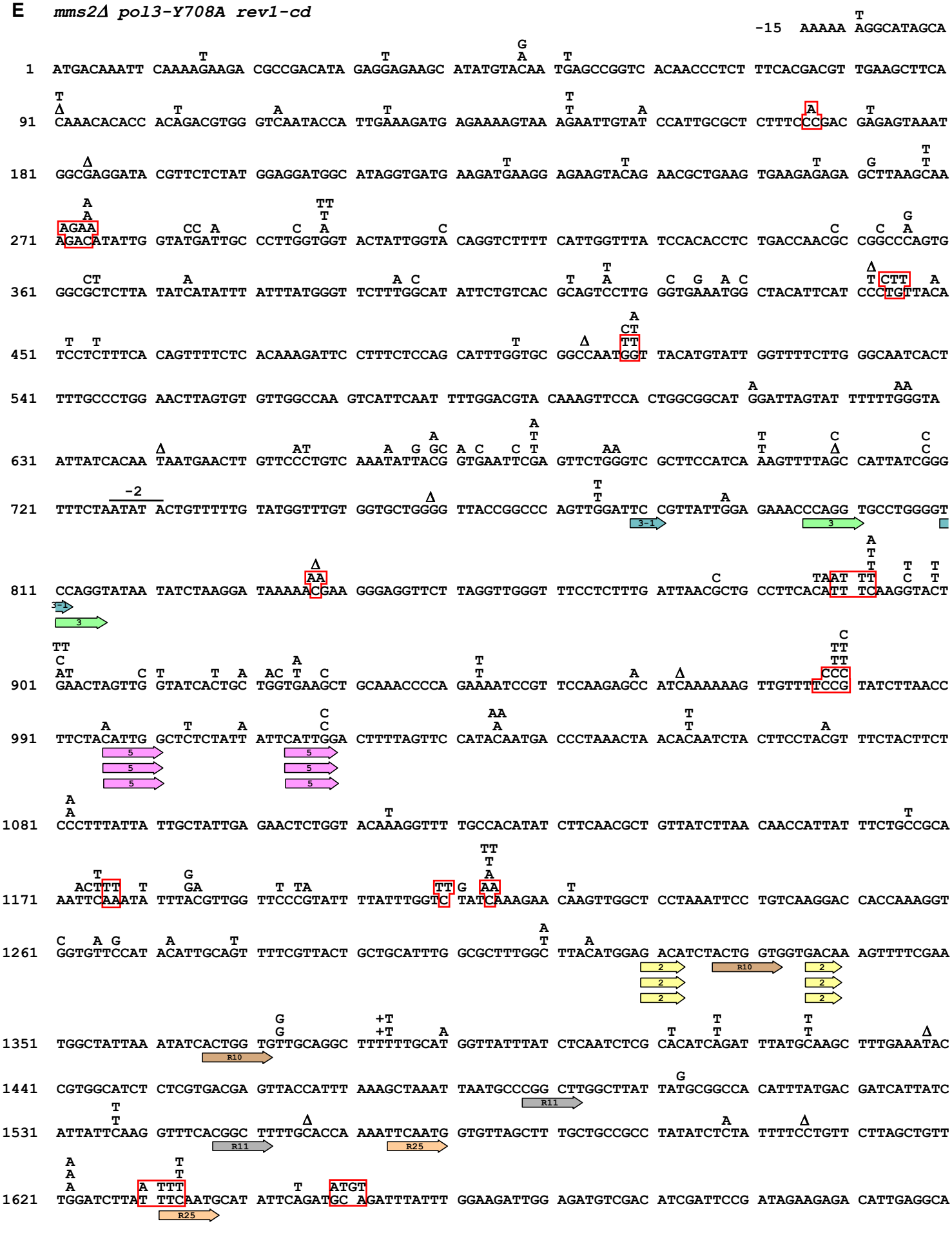
B *pol3-Y708A rev1-cd*



C *mms2Δ*



D *mms2Δ pol3-Y708A*



E *mms2Δ pol3-Y708A rev1-cd*