Supplemental Information.

Table S1. HSV-tk mutation frequencies and microsatellite specificity for Pol β WT and variants.

	HSV-tk Frequency x 10^{-4}		
Region	**WT**	**I260Q**	**Y265W**
$[G]_{10}$			
Observed	490 ± 21 (69)[a]	550 ± 250 (42)	760 (53)
Microsatellite	380 (32, 23)[b]	560 (27, 10)	140 (15, 19)
$[C]_{10\text{-}R}$			
Observed	280 ± 50 (44)	560 ± 56 (48)	1000 (43)
Microsatellite	300 (16,24)	690 (33, 8)	560 (11, 11)
$[C]_{10}$			
Observed	630 ± 230 (71)	1300 ± 400 (45)	830 (30)
Microsatellite	520 (4, 64)	1900 (3, 38)	580 (2, 19)
$[G]_{9}$			
Observed	190 ± 60 (28)	n.d.	n.d.
Microsatellite	200 (20,5)		
$[A]_{8}$			
Observed	400 ± 75 (80)[c]	480 ± 190 (30)	710 (33)
Microsatellite	390 (1, 76)	630 (1, 27)	470 (2, 20)
$[T]_{8}$			
Observed	380 ± 82 (81)[c]	1200 ± 350 (46)	1000 (54)
Microsatellite	350 (0, 74)	1100 (1, 37)	720 (6, 29)

[a] Mean of two or three experiments ± standard deviation. Number in parentheses is the total independent mutants isolated from two polymerase reactions.

[b] Number of (insertions, deletions)

[c] Data taken from Ananda et al. 2014 [43].

Table S2. Pol α-primase and η HSV-tk mutant frequencies and microsatellite specificity.

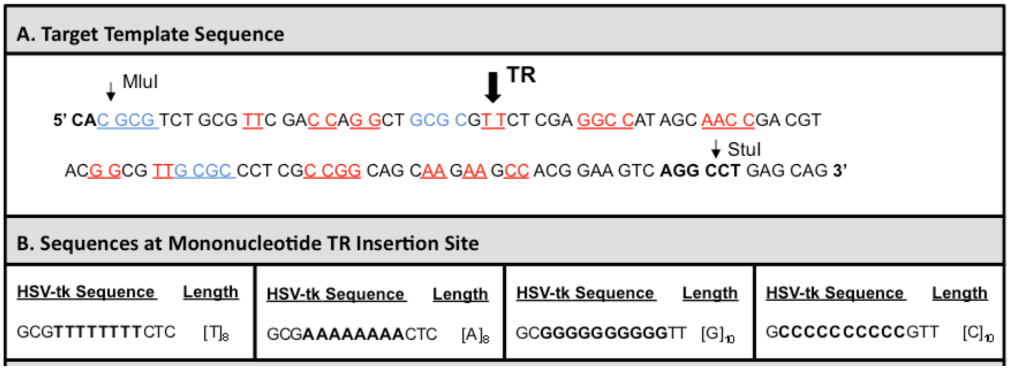
	HSV-tk Frequency x 10^{-4}	
Region	**Pol α-primase**	**Pol η**
[G]$_9$		
Observed	41 ± 19 (29)[a]	390 ± 99 (44)
Microsatellite	45 (3,26)[b]	200 (7,11)
[C]$_{10}$		
Observed	150 ± 6 (36)	800 ± 76 (46)
Microsatellite	140 (6,29)	320 (0,15)
[A]$_8$		
Observed[c]	55 (77)	260 (67)
Microsatellite	51 (7,64)	180 (0, 41)
[T]$_8$		
Observed[c]	47 (77)	640 (29)
Microsatellite	45 (13,61)	230 (1,15)

[a] Mean of two or three experiments ± standard deviation. Number in parentheses is the total independent mutants isolated from two polymerase reactions.

[b] Number of (insertions, deletions)

[c] Data taken from Ananda et al. 2014 [43].

Figure S1. Microsatellite constructs used in the HSV-tk experimental assay. A. Sequence of the experimental target sequence within the HSV-tk gene, from the MluI to StuI restriction sites. The large arrow shows the approximate position where the microsatellite (TR, tandem repeat) of interest is inserted in-frame within the target. Endogenous (HSV-tk-encoded) two-unit mononucleotide and dinucleotide repeats are shown in red and blue, respectively. **B.** Construction of the A_8, T_8, G_{10} and C_{10} mononucleotide-containing HSV-tk templates.



A. Target Template Sequence

5’ CAC GCG TCT GCG TTC GAC CAG GCT GCG CGT TCT CGA GGC CAT AGC AAC CGA CGT

ACG GCG TTG CGC CCT CGC CGG CAG CAA GAA GCC ACG GAA GTC **AGG CCT** GAG CAG **3’**

B. Sequences at Mononucleotide TR Insertion Site

HSV-tk Sequence	Length	HSV-tk Sequence	Length	HSV-tk Sequence	Length	HSV-tk Sequence	Length
GCG**TTTTTTTT**CTC	$[T]_8$	GCG**AAAAAAAA**CTC	$[A]_8$	GC**GGGGGGGGGG**TT	$[G]_{10}$	G**CCCCCCCCCC**GTT	$[C]_{10}$

Figure S2: Interruption errors within mononucleotide microsatellite alleles. All interruption mutations recovered from each template in all conditions are shown below. Insertions of bases other than the mononucleotide repeat are demarcated below the affected sequence with a black triangle with the inserted base shown below the triangle. Base substitution events are noted above the affected sequence with the identity of the substituted base. A. $[T]_8$ template, 20 µM dATP, 250 µM other three dNTPs. B. $[A]_8$ template, all four dNTPs 250 µM. C. $[A]_8$ template 20 µM dTTP, 250 µM other three dNTPs. *Indicates deletion of 6 As and base substitution of 2 Ts to As in one mutant. D. $[G]_9$ template, all four dNTPs 250 µM.