## RETROVIRAL MUTATION RATES AND REVERSE TRANSCRIPTASE FIDELITY

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### 1. ABSTRACT

Genetic variation in retroviral populations provides a mechanism for retroviruses to escape host immune responses and develop resistance to all known antiretroviral drugs. Retroviruses, like all RNA viruses, exhibit a high mutation rate. Polymerization errors during DNA synthesis by reverse transcriptase, which lacks a proofreading activity, is a major mechanism for generating genetic variation within retroviral populations. In this review, we summarize our current understanding of the processes that contribute to the generation of mutations in retroviruses. An overview of in vivo and in vitro studies of retroviral mutation rates determined by various fidelity assays is provided. Extensive mutational analyses of RTs are beginning to elucidate the relationship between structural determinants of RTs and fidelity of DNA synthesis. Recently, it was observed that the Y586F mutation in MLV RT results in a dramatic increase in the mutation rate in the vicinity of adenine-thymie tracts (AAAA, TTTT, and AATT), which are associated with bends in DNA. These results indicate that the templateprimer duplex is a component of the polymerase active site and its structure can influence nucleotide selectivity and the mutation rate. Additionally, the results also suggest that the Y586 residue and the RNase H primer grip are structural determinants of RT that have evolved to attenuate the effects of unusual conformations of the template-primer duplex, such as bends in DNA, on fidelity of DNA synthesis.

# 2. IMPORTANCE OF RETROVIRAL GENETIC VARIATION

All retroviral populations exhibit tremendous genetic variation that allows them to adapt to changes in their environment. Genetic variation has been documented extensively in populations of human immunodeficiency virus type 1 (HIV-1) (1-3). This genetic adaptability has significant consequences for the evolution of HIV-1 and other retroviruses, their impact on human health, and the ability of human societies to deal with the epidemic of acquired immunodeficiency syndrome (AIDS).

The genetic variation found in HIV-1 populations has allowed the virus to adapt by expanding its host range; for example, HIV-1 can switch from using the CCR5 coreceptor to using the CXCR4 coreceptor (4-6). Genetic variation is evident in the numerous HIV-1 clades that now infect various human populations and cause AIDS (7). The genetic variation between as well as within individual clades is a significant obstacle to the successful development of an anti-HIV-1 vaccine (8, 9). For HIV-1infected patients in the Western world, perhaps the most significant consequence of HIV-1 genetic diversity is the rapid development of resistance to antiretroviral drugs. Drug-resistant variants have arisen to over 116 antiretroviral agents that have been tested in the clinic or in the laboratory (10). Because of extensive genetic variation, it is expected that in response to any new antiretroviral agent, drug-resistant HIV-1 variants will emerge.

Genetic variation in retroviral populations is a consequence of the viral mutation rate (11-15, 16), recombination rate (17-21), rate of replication (1, 3), size of the viral population (22), and selective forces (2). In addition to a high rate of mutation, retroviruses also exhibit a high rate of recombination, which further increases variation in the viral population. Because the rates of viral mutation and recombination are considered as rates per cycle of replication, the number of replication cycles that occur per unit of time is an important factor that influences genetic variation in the viral population. The size of the viral population is also an important factor, because it sets an upper limit to the number of variants that can exist in the population at any given time. Of course, selective forces determine which viral variants will survive and contribute to the virion of the next generation (2).

In this review, we will outline the various mechanisms that affect the retroviral mutation rate. We will also summarize the current state of knowledge of the relationship between reverse transcriptase (RT) structure and its fidelity and discuss the influence of the templateprimer duplex structure on the fidelity of reverse transcription.

### 3. HISTORIC DEVELOPMENTS IN UNDERSTANDING RETROVIRAL GENETIC VARIATION AND RT FIDELITY

Peyton Rous observed genetic variation associated with retroviruses soon after the discovery of the Rous sarcoma virus (RSV) (23). He observed that some chicken sarcomas induced by the "filterable agent" were hemorrhagic, while others were composed of spherical rather than spindle-shaped cells. Although these variable characteristics could not be directly attributed to the viral genetic information, Duran-Reynals later found that certain variants of RSV, isolated from late-appearing tumors induced in with RSV, could infect ducks (24). Thus, genetic variants of RSV with different biological properties were identified.

Howard Temin developed a quantitative assay for RSV (25) and used it to perform a detailed study of

variation associated with RSV (26). He noted that infection of chick embryo fibroblasts *in vitro* resulted in formation of foci with distinct morphological phenotypes. Clonal stocks of virus derived from a focus produced foci with the same morphology, indicating that the focus morphology was, at least in short-term cultures, a genetic characteristic of the virus.

Soon after the discovery and isolation of RT (27, 28), it was noted that this DNA polymerase exhibited a high error rate when it is used to copy homopolymeric RNA and DNA templates (29-32). In the first reported measurement of RT fidelity, the mutation rate of avian myeloblastosis virus (AMV) RT was estimated to be approximately 1 in 600 for dCMP incorporation on a poly A template (29). These observations led to the suggestion that under certain conditions of DNA polymerization, RT makes a significant number of errors that might play a role in spontaneous mutation (32). Later, Coffin et al. estimated the mutation rate of a specific nucleotide position to be approximately  $10^{-4}$  mutations per replication cycle in a continuously passaged culture of RSV (33).

Gojobori and Yokoyama (34) compared the rate at which mutations accumulated in the retroviral v-mos oncogene in a Moloney murine sarcoma virus (MoMSV) and its cellular homolog, c-mos. They found that the rate of mutation accumulation in v-mos was approximately a million-fold higher than the rate of mutation accumulation in c-mos ( $1.3 \times 10^{-3}$  vs.  $1.7 \times 10^{-9}$  per site per year, respectively). These results established that retroviruses evolve much more rapidly than mammalian organisms as well as DNA-containing microbes. However, their rates of evolution are similar to those of other RNA viruses (35). All RNA viruses, including retroviruses, appear to exhibit a high degree of variability and are described as a quasispecies, which is characterized as a large collection of genetically related but not identical genomes (36).

The development of sensitive genetic assays that measure either the reversion of a nonsense codon (37) or forward mutations that inactivate a reporter gene (38) has greatly facilitated measurements of the error rates of RTs (39). Additionally, accurate measurements of the *in vivo* retroviral mutation rates have been made possible by the development of retroviral vectors and packaging cell lines that allow the virus to undergo a single cycle of replication in a controlled manner (40, 41).

# 4. FACTORS THAT INFLUENCE RETROVIRAL MUTATION RATE

## 4.1. Reverse transcription

Mutations may be introduced into retroviral genomes during various steps in the viral life cycle (figure 1). After the viral RNA enters the cytoplasm of the target cell, it is first copied in an RNA-dependent DNA synthesis step to generate a minus-strand DNA, which is subsequently copied in a DNA-dependent DNA synthesis step to generate a double-stranded DNA form (42). RTs, which carry out these two polymerization steps, lack exonucleolytic proofreading activity and thus rely only on



Figure 1. Retroviral replication cycle. Virion core containing two RNA copies of the retroviral genome enters the cytoplasm. RT copies Viral RNA (thin line) into minus-strand DNA (thick line) in a step labelled RNAdependent DNA synthesis. For simplicity, only one RNA is shown. Next, RT copies minus-strand DNA into doublestranded DNA (thick lines) in a step labeled DNAdependent DNA synthesis. The fidelity of both RNA- and DNA-dependent DNA synthesis may be influenced by intracellular dNTP pools. Polymerization errors that occur DNA DNA-dependent synthesis during form heteroduplexes that may be subjected to DNA repair. Integration of the viral DNA into the cell chromosome forms the provirus. Proviral DNA is replicated by mammalian DNA polymerases during each cell division. Viral RNA is synthesized by cellular RNA polymerase II, which uses the integrated provirus as a template. Translation and assembly of viral proteins is followed by formation of viral particles at the plasma membrane. Steps of retroviral replication cycle and factors that could influence the rate of viral mutation are indicated in bold type.

discrimination against the incorrect incoming nucleotide during polymerization to prevent errors. Consequently, polymerization errors could occur during either RNAdependent or DNA-dependent DNA synthesis.

## 4.2. Other viral proteins

RT is not the only viral protein that influences the retroviral mutation rate. Several retroviruses encode a dUTPase that suppresses the incorporation of uracil into the viral genome and increases the fidelity of reverse transcription (43-48). Mutations in the HIV-1 accessory protein Vpr have been shown to influence the HIV-1 mutation rate by interacting with uracil DNA glycosylase and facilitating its incorporation into virion (49). Recent studies have shown that the nucleocapsid protein (NC) can enhance the rate of viral DNA synthesis in regions of the template containing secondary structure (50); the results also suggest that murine leukemia virus (MLV) NC could have a significant impact on the viral mutation rate.

## **4.3.** Nucleotide pools, DNA repair, and mammalian DNA polymerases

In addition to viral proteins, the intracellular environment, mammalian polymerases, and nucleic acid modifying enzymes could potentially influence the retroviral mutation rates. Alteration of the intracellular nucleotide pools has been shown to increase the retroviral mutation rates (51).

Mutations that occur during DNA-dependent DNA synthesis result in the formation of heteroduplexes that could be potentially recognized by the host DNA repair enzymes and corrected in a strand-specific manner to influence the overall mutation rate. Recent studies have indicated that mismatches involving large loops can be efficiently repaired by the host repair system, which could affect the overall mutation rate (52). Mutations can also occur during replication of the integrated provirus through cell division. However, host cell DNA polymerases have mutation rates  $(10^{-9} to 10^{-12} mutations/basepairs (bp)/cycle)$  that are significantly lower than the mutation rates of RTs and their contribution to the retroviral mutation rates is probably negligible (53).

## 4.4. RNA transcription

Perhaps the most significant contribution to retroviral errors by a non-viral mechanism is polymerization errors during RNA polymerase II- mediated transcription. The mutation rate of RNA polymerase II has not been accurately determined, and thus its contribution to the retroviral mutation rate is unknown. Analysis of mutations that occur in a reporter gene inserted in the long terminal repeat (LTR) has indicated that approximately 1/3 of the mutations occur during the DNA-dependent DNA synthesis step of reverse transcription and the remaining 2/3 of the mutations occur during RNA transcription and the RNA-dependent DNA synthesis step of reverse transcription (54). These results provide an upper limit to the contribution of RNA polymerase II to retroviral genetic variation: assuming that the error rates of the three polymerization steps (RNA transcription, RNA-dependent DNA synthesis, and DNA-dependent DNA synthesis) are similar, the mutation rate of RNA polymerase II is around  $0.5-1 \ge 10^{-5}$  mutations/bp/replication cycle. This estimate is at the lower end of the *in vitro* mutation rate measured for wheat-germ RNA polymerase II (10<sup>-3</sup> to 10<sup>-5</sup> mutations per/bp/cycle) (55).

Eukaryotic as well as bacterial RNA transcription processes appear to possess a proofreading ability, suggesting that RNA transcription may be more accurate than reverse transcription. Eukaryotic transcription factor SII and bacterial GreA and GreB proteins have been shown to stimulate the excision of misincorporated bases from transcribed RNA (56-58). A recent study suggested, however, that the rate of translational errors is significantly higher than that of transcriptional errors in yeast strains lacking SII, suggesting that any proofreading activity provided by SII is unlikely to be physiologically relevant (59).



Figure 2. In vivo assays for measurement of retroviral mutation rates in a single replication cycle. A. Retroviral vector containing LTRs, packaging signal (psi), and a reporter gene is shown. Commonly used reporter genes are lacZ, lacZa, GFP, and HTK. B. A producer cell clone containing an integrated provirus derived from a retroviral vector is shown. Proviral DNA is transcribed by RNA polymerase II to generate viral RNA that is packaged into infectious viral particles. Virion produced from the producer cells are used to infect target cells. Upon infection, reverse transcription of the viral genomic RNA occurs and involves one cycle of RNA-dependent DNA synthesis and one cycle of DNA-dependent DNA synthesis. Mutations that inactivate the reporter gene during RNA transcription or reverse transcription can be identified phenotypically and characterized by DNA sequencing.

Slippage-induced frameshift errors induced by mammalian RNA polymerase II were analyzed by employing an apoB mutant allele containing a deletion of a single cytosine, creating a stretch of eight adenines. It was demonstrated that transcriptional slippage occurs with a frequency of 10% by the insertion of an extra adenine into the stretch of eight adenines (60). A similar transcriptional slippage frequency of 25-30% was also documented for the *Escherichia coli* (*E. coli*) RNA polymerase during elongation at stretches of ten or more adenines or thymines, which were detected by restoration of the proper reading frame of the bacterial beta-galactosidase (*lacZ*) reporter construct (61).

### 4.5. RNA modification

Another potential mammalian host cell mechanism that contributes to retroviral genetic variation is RNA modification. The host cell double-stranded RNA adenosine deaminase (dsRAD) can modify retroviral RNAs by deamination of adenosines to inosines, which ultimately result in A-to-G hypermutation of the viral genomes (54, 62, 63). However, the low frequency of A-to-G hypermutation in retroviral sequences suggests that RNA modification of retroviral genomes occurs rarely.

#### 4.6. Antiviral nucleoside analogs

Treatment with the 3<sup>-</sup>-azido-3<sup>-</sup>-deoxythymidine (AZT) profoundly increases the retroviral mutation rate in an RT-dependent manner (64). The mutation rate of spleen necrosis virus (SNV) was increased seven- to tenfold in the presence of AZT while similar concentrations of AZT resulted in only a two- to threefold increase in the MLV mutation rate (64). The mutation rate of FIV was increased in the presence of AZT approximately threefold (65). Later, it was shown that AZT also increased the mutation rate of HIV-1 about eightfold (66). Interestingly, 2',3'-dideoxy-3'-thiacytidine (3TC) also increased the mutation rate modestly by threefold. The simple hypothesis that AZT competes with intracellular nucleosides for phosphorylation and thereby alters the intracellular nucleotide pools was not supported by experimental evidence (64). Thus, the mechanism by which AZT increases the mutation rate of RTs is unclear.

## 5. IN VIVO FIDELITY ASSAYS AND MUTATION RATES

#### 5.1. In vivo fidelity assays

A generalized approach to measuring in vivo retroviral mutation rates is outlined in figure 2. A retroviral vector encoding a reporter gene is constructed. Typically, the product of a mutation reporter gene is easily identifiable phenotypically or can be selected. The *lacZ* or its truncated peptide (acZa), herpes thymidine kinase gene (HTK), neomycin phosphotransferase gene (neo), and green fluorescent protein gene (GFP) have been used as mutation reporters. The retroviral vector encoding the reporter gene is introduced into a packaging cell line and the virus produced is used to infect target cells: the vector can complete one round of replication and integrate in the target cell genome to form a provirus. However, because the vector is unable to express any viral proteins, additional cycles of replication cannot occur. A single cycle of retroviral replication constitutes one cycle of RNA transcription by RNA polymerase II, one cycle of RNAdependent DNA synthesis, and one cycle of DNAdependent DNA synthesis.

The assays can be designed to detect the inactivation of the reporter gene (forward mutation assays) or to detect the reversion of an inactivating mutation introduced in the reporter gene (reversion assays) (13, 67). Because the forward mutation assays provide an average mutation rate of several hundred target nucleotides, the observed mutation rate is likely to be representative of the mutation rate of viral genes and sequences. In contrast, only one or a few nucleotide targets are monitored in the reversion assays; because retroviral mutation rates are highly sequence dependent and mutational hotspots and coldspots have been well-documented, the target nucleotides chosen in a reversion assay may or may not reflect the overall mutation rate.

The *in vivo* forward mutation rates represent the most reliable measurements of retroviral mutation rates because they are determinations of polymerization errors that occurred in the context of a replicating complex composed of all of the viral proteins under *in vivo* conditions of pH and nucleotide concentrations. However, the *in vivo* forward mutation assays also have their limitations. First, not all mutations in the reporter gene result in a detectable phenotypic change in the gene product; thus, estimates of the mutation rate depend on assumptions made about

RT	Reporter gene	Mutation Rate (* 10 <sup>-5</sup> mutations/bp /cycle)	Reference
HIV-1	LacZa	3.4 <sup>a</sup>	14
SNV	LacZa	1.1	12, 13
SNV	LacZa	2.2	54
SNV	Neo	500	71
	(reversion)		
SNV	Neo	2.0	67
	(reversion)		
BLV	LacZa	0.5	16
HTLV-1	LacZa	0.7	15
RSV	viral	14 <sup>b</sup>	114
	genome		
Ty1	Tyl	2.5 <sup>c</sup>	69
MLV	LacZ	2.5 <sup>d</sup>	84, 85,104
MLV	GFP	2.1 <sup>e</sup>	104
MLV	HTK	1.6 <sup>f</sup>	86
MLV	Neo	0.2	77
	(reversion)		

<sup>a</sup> Mutation rate was calculated based on the predetermined number of mutational targets for *lacZa* reporter gene (113 bp).<sup>b</sup> Presence of mutations in viral sequences was detected by denaturing gradient gel electrophoresis. <sup>c</sup> Mutation rate was calculated as follows:  $(13/510,777 = 2.5 \times 10^{-5})$  number of detected substitutions (13) was divided by number of replicated nucleotides (29 transpositions x 17,613 nt per replication cycle = 510,777 nt).<sup>d</sup> Mutation rate was calculated based on estimated number of mutational targets as 2/3 of the length of lacZ reporter gene. Mutation rate was determined as follows: (5.2% mutant frequency divided by number of mutational targets 2040 bp =  $2.5 \times 10^{-5}$ ). <sup>e</sup> Mutation rate was calculated based on estimated number of mutational targets as 2/3 of the length of GFP reporter gene. Mutation rate was determined as follows: (1.0% mutant frequency divided by number of mutational targets 472 bp =  $2.1 \times 10^{-10}$ <sup>5</sup>). <sup>f</sup> Mutation rate was determined only for the mutations that involved large genetic rearrangements. An approximate overall mutation rate is calculated by estimating the number of mutational targets as 2/3 of the length of HTK reporter gene (1747 of 2620 bp total) as follows: (8.8% mutant frequency divided by number of 1747 bp = 5.0 x mutational targets 10-5 mutations/bp/cycle).

the number of mutational targets present in the reporter gene that reliably result in a detectable mutant phenotype. Detailed information based on experimental data of mutational target sites is available for the *lacZa* gene (113 target sites for a total length of 280 nucleotides) and the measured rates are likely to be accurate (68). However, the numbers of mutational targets are not known for the complete *lacZ* gene, HTK, or GFP. A second potential limitation is that the mutation rates that are measured using reporter genes may not be representative of the viral genes and sequences. Because forward mutation rates represent an average of several hundred target sites, they are likely to be representative of viral sequences as well. The mutation rate of the Ty1 transposable element was measured by direct sequencing of 173,043 nucleotides and the rate was determined to be  $2.5 \times 10^{-5}$  mutations/bp/cycle (69). This result suggests that mutation rates based on direct sequencing of viral genomes are likely to be similar to those measured by using reporter gene-based assays. However, the possibility that viral sequences have evolved to minimize the impact of RT mutations cannot be ruled out. A third limitation is that the observed mutation rates represent the sum of the RT mutation rate and the RNA polymerase II mutation rate; thus, the RT mutation rate cannot be directly measured.

### 5.2. *In vivo* mutation rates

The in vivo forward mutation rates have been measured for SNV, HIV-1, bovine leukemia virus (BLV), human T cell leukemia virus type 1 (HTLV-1), MLV, RSV, and the Ty1 retroelement (table 1). With the exception of RSV, these mutation rates are very similar to each other and range from 0.5 x 10<sup>-5</sup> mutations/bp/cycle for BLV to  $3.4 \times 10^{-5}$  mutations/bp/cycle for HIV-1. It is important to point out that the HIV-1 in vivo mutation rate is within twofold of the MLV and SNV mutation rates; even though HIV-1 appeared to be substantially more error-prone in some studies, its *in vivo* mutation rate is very similar to that of gammaretroviruses (11, 70). Most of the in vivo mutation rates observed to date are within a threefold range of an average of 1.5 X 10<sup>-5</sup> mutations/bp/cycle. The only exception is the high mutation rate of RSV (14 X 10<sup>-5</sup> mutations/bp/cycle), which was measured using viral sequences as a target and denaturing gradient gel electrophoresis. Because the method used to measure the RSV mutation rate was significantly different from the method used for other viruses, the rates may not be directly comparable.

The *in vivo* forward mutation rate of MLV was determined using several reporter genes (*acZ*, GFP, and HTK) and the observed rates were similar, suggesting that sequence differences among reporter genes do not significantly affect fidelity and mutation rate estimates. There were exceptions, however, indicating that the method of measurement of the *in vivo* mutation rates could be important for obtaining an accurate result. For example, *in vivo* reversion assays using the *neo* reporter gene displayed two mutation rates for SNV RT that differ by 250-fold (67, 71).

# 6. IN VITRO FIDELITY ASSAYS AND MUTATION RATES

### 6.1. In vitro fidelity assays

In vitro assays can be used to measure the mutation rate of purified RT in the presence of nucleotide substrates and a template-primer complex. As discussed earlier, the *in vitro* assays can also be set up to measure the forward mutation rate of a reporter gene or reversion of a nonsense codon. A forward mutation assay in which the *lacZa* gene serves as a mutation reporter is frequently used to measure the mutation rates of purified RTs (11, 72-75). In this assay, a gapped-duplex DNA is generated from a genetically engineered single-stranded bacteriophage,

M13mp2; the gapped single-stranded DNA is copied by RT in the presence of dNTP substrates, and errors that occur during this synthesis are quantified by analyzing the phenotype of plaques generated by infection of host bacteria.

In misinsertion assays, a binary complex is formed between the RT and a template-primer (70, 76-78). Then, the ability to extend the primer in the presence of the correct and incorrect nucleotide substrate is determined. The efficiency of primer elongation is measured by quantitative gel electrophoresis; the data are analyzed using the Michaelis-Mention equation and the parameters  $K_{cat}$ and  $K_m$  are determined for the correct and incorrect nucleotide substrate. Misinsertion efficiency ( $F_{ins}$ ) is defined as the ratio of  $K_{cat}/K_m$  for the incorrect nucleotide divided by the  $K_{cat}/K_m$  for the correct nucleotide. The rate of polymerization ( $K_{cat}$ ) should be higher for the correct nucleotide than for the incorrect nucleotide; on the other hand, the affinity of RT for the correct nucleotide should be higher (lower  $K_m$ ) than for the incorrect nucleotide.

In mismatch extension assays, template-primers in which the 3' terminus of the primer strand is correctly matched to the template or is mispaired are used (76-78). The kinetics of extending the mismatched primer are compared with the kinetics of extending the correctly matched primer. The mismatch extension ratio ( $F_{ext}$ ) is  $K_{cat}/K_m$  for the mismatched primer divided by the  $K_{cat}/K_m$ of the correctly matched primer.

The in vitro fidelity assays have the advantage that they can be performed under defined conditions. RT fidelity can be measured on either the RNA or DNA template without the complication of errors introduced during RNA transcription. Misincorporation occurs through a series of steps that include discrimination of the correct and incorrect nucleotide substrate, the incorrect nucleotide binding to the substrate-binding site, phosphate bond formation, and extension of the mismatched nucleotide. In vitro assays have the potential to dissect and analyze these various steps in detail. However, like in vivo assays, in vitro assays also have limitations. First, the conditions of the assay such as pH, nucleotide concentrations, the nature and concentration of the divalent cation, and the nature of the template-primer can all significantly impact the observed mutation rate (79-83). Second, certain conditions such as the stability and structure of the template-primer complex and the ratio of RT to template-primer complex may impact the observed results. One concern about the misinsertion assays is the potential contribution of contaminating nucleotides to the primer extension that appears as misinsertion. Third, the limitations of a codon reversion assay also apply to these assays, because the misinsertion and mismatch extension rates at only one or a few target nucleotides, which may or may not represent the overall rate are measured. Finally, perhaps the most important drawback is that the potential influence on the mutation rate of other viral proteins, the structure of the reverse transcription complex, and other aspects of the intracellular environment such as the balance of endogenous nucleotide pools are not taken into account.

### 6.2. *In vitro* mutation rates

The in vitro forward mutation rate for HIV-1 RT has been determined using the *lacZa* reporter gene by several investigators (11, 72, 74, 75) (table 2). The reported mutation rates range from 5.3 x 10<sup>-5</sup> mutations/bp/cycle to 59 x  $10^{-5}$  mutations/bp/cycle. These mutation rates are up to 17-fold higher than the in vivo forward mutation rate determined using the same mutation reporter gene (11, 14). A comparison of the sites of mutations in vivo and in vitro indicates that the locations of the mutations as well as their rates vary widely between the *in vitro* and *in vivo* assays (14). Furthermore, a comparison of the mutational hotspots in the *lacZa* gene determined in three separate studies suggests that the sites and nature of mutations can be dependent on the conditions of the assay (72, 74, 75). These results have suggested that there are elements of the *in vivo* conditions that are missing from the in vitro assays and that these factors can greatly influence the fidelity of DNA synthesis.

Despite these caveats, the *in vitro* forward mutation assays for MLV RT have provided mutation rates that are similar to each other and to the *in vivo* forward mutation rates (72, 73, 84-86). A comparison of the *in vitro* forward mutation rates of SIV, AMV, and MLV RTs suggest that the mutation rates of these RTs are similar (72, 73, 75).

The mutation rates determined by the misinsertion and mismatch extension assays are quite variable, suggesting that they are highly dependent on the conditions of the assay. For HIV-1 RT, the range of misinsertions is approximately 2200-fold, varying from  $0.02 \times 10^{-5}$  mutations/bp/cycle to 44 x  $10^{-5}$ mutations/bp/cycle (78, 87-89). The rates for HIV-1 RT mismatch extension vary from 10 x 10<sup>-5</sup> mutations/bp/cycle to 590 x 10<sup>-5</sup> mutations/bp/cycle (78, 90, 91). Varela-Echavarria et al. compared the MLV rate of mutation determined in vivo for a single nucleotide position (0.2 x 10<sup>-5</sup> mutations/bp/cycle) with the rate of misinsertion for the identical nucleotide sequence (77). They found that the A-C mismatch occurs at a rate comparable to the *in vivo* mutation rate (0.4 x  $10^{-5}$  mutations/bp/cycle) but the T-G mismatch occurs at a rate that is 30-fold higher (7 x 10<sup>-5</sup> mutations/bp/cycle). Again, these results suggest that additional factors that improve the fidelity of reverse transcription are present in the infected cells that are absent from the in vitro assays.

## 7. SPECTRUM OF MUTATIONS AND THEIR RELATIVE FREQUENCIES

SNV, MLV, and HIV-1 RTs induce a similar broad spectrum of mutations during reverse transcription *in vivo* (12-14, 86, 92). Approximately 51–81% of the mutations characterized are substitution mutations. Among the substitution mutations, approximately 80% are transitions and 20% are transversions, and G-to-A transitions are generally the most frequent. About 10-25% of the mutations are frameshift mutations that occur in stretches of identical nucleotides; increasing the length of the stretches of nucleotides dramatically increases the

Table 2. In	vitro retroviral	mutation rates
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RT	Assay – Reporter gene or	Mutation Rate	Reference
	nucleic acid template	(* 10 <sup>-5</sup> mutations/bp/cycle)	
HIV-1	In vitro-FMA <sup>a</sup> – lacZa	59	11
HIV-1	In vitro-FMA – lacZa	16	74
HIV-1	In vitro-FMA – lacZa	17	72
HIV-1	In vitro-FMA – lacZa - RNA	14	72
HIV-1	In vitro FMA – lacZa	5.3	75
HIV-1	In vitro FMA – EnvlacZa <sup>b</sup>	1.8	75
HIV-1	In vitro-FMA – HIV-1env	19	115
HIV-1	In vitro-FMA – HIV-1env-RNA	20	115
HIV-1	Misinsertion – DNA	0.6 - 66.6	70
HIV-1	Misinsertion – DNA	4.4 - 16	78
HIV-1	Misinsertion – DNA	6 - 57	87
HIV-1	Misinsertion – DNA	1.4 - 28.9	89
HIV-1	Misinsertion – DNA	0.2 - 125	88
HIV-1	Misinsertion – RNA	15 - 67	78
HIV-1	Misinsertion – RNA	0.02 - 8.3	88
HIV-1	Mismatch extension – DNA	4.1 - 38.4	91
HIV-1	Mismatch extension – DNA	10 - 76.9	116
HIV-1	Mismatch extension – DNA	140 - 590	78
HIV-1	Mismatch extension – RNA	550 - 2700	78
BLV	Misinsertion – DNA	0.3 - 8.6	117
BLV	Mismatch extension – DNA	19.6 - 29	117
Ty1	Misinsertion – DNA	0.5 - 6.3	118
Ty1	Misinsertion – RNA	0.8 - 1.7	118
MLV	In vitro FMA – lacZa	3.3	73
MLV	In vitro FMA – lacZa	3.4	72
MLV	In vitro FMA – lacZa - RNA	2.7	72
MLV	In vitro FMA – EnvlacZa	1.9	75
MLV	Misinsertion – DNA	0.4 - 6.7	77
MLV	Mismatch extension – DNA	0.5 - 9.1	91
MLV	Mismatch extension – DNA	80 - 150	77
MLV	Mismatch extension – RNA	5.3 - 33.3	91
SIV	In vitro FMA – EnvlacZa	1.1	75
SIV	In vitro FMA – lacZa	2.9	75
SIV	Misinsertion – DNA	0.4	119
SIV	Misinsertion – RNA	2.7	119
AMV	In vitro FMA – lacZa	3.3	73
AMV	Misinsertion – DNA	0.3 - 3.8	70
AMV	Misinsertion – DNA	16 - 59	78
AMV	Misinsertion – DNA	0.3 - 4.7	120
AMV	Misinsertion – DNA	0.6 - 2.4	118
AMV	Misinsertion – RNA	3.2 - 30	78
AMV	Mismatch extension – DNA	56 - 5800	78
AMV	Mismatch extension – RNA	210 - 4500	78
AMV	Mismatch extension – DNA	0.3 - 23	120
HIV-2	Mismatch extension – DNA	0.5 - 34.4	106
HIV-2	Mismatch extension – DNA	2.9 - 55.5	91
HIV-2	Mismatch extension – RNA	8.3 - 71	91
MMTV <sup>c</sup>	Misinsertion – DNA	0.9 - 10.1	120
MMTV	Mismatch extension – DNA	0.3 - 11.2	120
$EIAV^{d}$	Misinsertion – DNA	1.2 - 33.3	89
EIAV	Mismatch extension – DNA	2.8 - 25	89
EIAV	Mismatch extension – RNA	7 - 47	89

<sup>a</sup> FMA, forward mutation assay. <sup>b</sup> *EnvlacZa*, fusion product of the fragment of SIV envelope and *lacZa* (75). <sup>c</sup> MMTV, mouse mammary tumor virus. <sup>d</sup> EIAV, equine infectious anemia virus.

frequency of frameshifts, which are believed to occur through a slippage mechanism (12, 93, 94). The remaining 10-25% of the mutations occur by RT switching templates from one region of the template to another, which results in simple deletions and deletions with insertions; simple deletions occur through template switching events



**Figure 3.** Structure of HIV-1 RT bound to a DNA-DNA template-primer complex. Representation of the HIV-1 RT bound to template-primer complex and incoming dTTP was generated based on RT structure described in reference (98) using the Ras-Mol program. The fingers, palm, thumb, and RNase H domain of the p66 subunit are indicated. The A-form and B-form DNAs of the template-primer complex are labeled. A 41-degree bend is present at the A-form/B-form junction.

involving short direct repeats at the deletion junctions, whereas deletions with insertions involve more complex template switching events. On rare occasions, G-to-A hypermutations are observed in which multiple substitutions occur within the same viral genome; the mechanism by which these mutations occur is unknown but may involve reverse transcription by highly error-prone polymerases or by biased nucleotide pools (13, 95). Other infrequent mutations involving duplications by RT template switching and A-to-G hypermutations by dsRAD have been reported (54).

## 8. STRUCTURAL DETERMINANTS OF RT THAT INFLUENCE FIDELITY

#### 8.1. Structure of RT

The structure of RT is likely to be responsible for its low fidelity of DNA synthesis. As already mentioned, RT lacks the exonucleolytic proofreading activity that is a feature of most cellular DNA polymerases. It was hypothesized that because two template-switching events (called minus-strand transfer and plus-strand transfer) are necessary for the completion of reverse transcription, retroviral RTs evolved to possess low template affinity and low processivity (96). The template-switching property of RTs results in additional intramolecular and intermolecular template-switching events that lead to formation of deletions and recombination.

The structure of RT is likely to play an important role in its low template affinity and low processivity. Several crystal structures of HIV-1 RT have been determined, including cocrystals with nonnucleoside inhibitors, a DNA:DNA template-primer hybrid, an RNA:DNA hybrid, and a ternary complex with DNA and dTTP substrate (97-101). HIV-1 RT is a heterodimer composed of p66 and p51 subunits (figure 3). The p66 subunit possesses both polymerase and RNase H activities. The structure of RT is often compared to a right hand, and the various domains of RT are referred to as fingers, palm, thumb, connection, and RNase H. The p51 subunit lacks the RNase H domain and is folded in a different conformation.

A partial MLV RT crystal structure has been solved for the N-terminal segment of the protein containing the fingers and palm domains (102, 103). Despite a low primary sequence homology between HIV-1 and MLV RTs, the three-dimensional structures of the fingers and palm domains appear to be similar (102).

#### 8.2. Structural determinants of in vivo fidelity

To date, few studies have analyzed the effects of mutations in RTs on the in vivo fidelity of reverse transcription (15, 66, 84, 85, 104) (table 3). Halvas et al. have performed extensive mutational analysis of MLV RT and determined the effects of the mutations on the *in vivo* fidelity of reverse transcription (84, 85). In the first study, mutational analysis of the V223 residue of the conserved YXDD catalytic site motif indicated that substitution with methionine, the residue found at the equivalent 184 position in HIV-1 RT, resulted in a 1.8-fold increase in the mutation rate. Mansky et al. made a similar observation that the opposite substitution in HIV-1 RT (M184V), which is associated with resistance to the antiviral drug 3TC. resulted in a 1.3-fold decrease in the mutation rate (66). Mansky and colleagues have also determined the effects of HIV-1 RT mutations that confer resistance to AZT on the

Mutation	Description	Reporter gene	Relative mutant frequency	Reference
HIV-1	-		* •	
M184V	YMDD/3TC <sup>Ra</sup>	lacZa	↓ 1.3X	66
T215Y	AZT <sup>Rb</sup>	lacZa	↑1.3X	66
M41L/	AZT <sup>R</sup>	lacZa	↑3.3X	66
T215Y				
M41L/	AZT <sup>R</sup>	lacZa	14.3X	66
D67N/				
K70R/				
T215Y				
HTLV-1				
M188A	YMDD	lacZa	↑ 2.6X	15
M188V	YMDD	lacZa	↑ 2.3X	15
MLV				
K103R	Fingers/dNTP binding	lacZ	No Change	84
T147A		lacZ	↑ 1.3 X	84
L151F		lacZ	↑ 2.4 X	84
K152A		lacZ	↑ 1.4 X	84
D153A	dNTP binding	lacZ	↑ 1.6 X	84
D153C	dNTP binding	lacZ	No Change	84
D153Q	dNTP binding	lacZ	No Change	84
D153S	dNTP binding	lacZ	No Change	84
A154S		lacZ	↑ 1.3 X	84
F155W	dNTP binding	lacZ	↑ 2.8 X	84
F155Y	dNTP binding	lacZ	No Change	84
F156L		lacZ	↑ 1.6 X	84
F156M		lacZ	↑ 1.7 X	84
F156W		lacZ	↓ 1.3 X	84
F156Y		lacZ	↓ 1.3 X	84
C157A		lacZ	↑ 1.3 X	84
R159A		lacZ	No Change	84
H161A		lacZ	↑1.2 X	84
Q190M	dNTP binding	lacZ	No Chg.	84
V223A	Palm/YVDD	lacZ	1.7 X	85
V223I	Palm/YVDD	lacZ	No Change	85
V223M	Palm/YVDD	lacZ	↑ 1.8 X	85
V223S	Palm/YVDD	lacZ	↑ 2.3 X	85
S526A	RNase H	lacZ	↑ 1.6 X	85
Y586F	RNase H	lacZ	↑ 5.4 X	104
		GFP	↑4.3 X	104
Y598V	RNase H	lacZ	No Change	85
R657S	RNase H	lacZ	↑1.4 X	85

**Table 3.** Structural determinants of *in vivo* fidelity

<sup>a</sup> 3TC<sup>R</sup>, mutation confers resistance to 3TC. <sup>b</sup> AZT<sup>R</sup>, mutation confers resistance to AZT.

accuracy of DNA synthesis. In general, mutations that conferred resistance to AZT increased the mutation rate; the largest increase in the mutation rate, 4.3-fold, was quadruple observed with а mutant (M41L/D67N/K70R/T215Y) (66). Extensive mutational analysis of MLV RT dNTP-binding site residues was performed to determine their effects on fidelity (84). Substitution of F155, which contacts the base and ribose moiety of the substrate dNTP, with tryptophan increased the mutation rate 2.8-fold. Interestingly, substitution L151F, which is adjacent to the catalytic site residue D150, resulted in a 2.4-fold increase in the forward mutation rate.

Most single amino acid substitutions resulted in a less than 3-fold change in the *in vivo* forward mutation rate.

The only exceptions were the triple and quadruple mutations that conferred resistance to AZT and increased the mutation rate 3.3- and 4.3-fold, respectively (66). Therefore, it was surprising that in a recent study a 5.4-fold increase was observed in the *in vivo* forward mutation rate of *lacZ* that resulted from a single amino acid substitution (Y586F) in the RNase H primer grip motif of MLV RT (104).

These studies have identified several different structural elements of RTs as important determinants that maintain the accuracy of DNA synthesis. Their overall influence on fidelity is to increase the *in vivo* mutation rate approximately two- to fivefold. The YXDD catalytic site motif, mutations that confer resistance to AZT, dNTP- binding site, and the RNase H primer grip motif appear to influence the *in vivo* accuracy of reverse transcription (66, 84, 85, 104).

### 8.3. Structural determinants of *in vitro* fidelity

The effects of HIV-1 RT mutations on accuracy of DNA synthesis has been determined for several mutations by using an *in vitro* forward mutation assay in which the *lacZa* gene was used as the mutation reporter (table 4). In addition, mutational analyses of MLV and HIV-2 RTs were reported (105, 106). Of the mutations that have greater than twofold effects on fidelity, a majority increased the accuracy of DNA synthesis (9 of 12). Whether this bias reflects the nature of mutations that have been tested to date or the conditions of the assay, such as nucleotide concentrations or the use of only a DNA template, is unknown at this point. A cluster of four mutations in the fingers domain (F61A, K65R, D76V, and R78A) increased the accuracy of DNA synthesis 9- to 12fold, and the L74V substitution increased the accuracy by 3.4-fold in one study. Two dNTP binding site mutants. Y115V and Q151N, increased fidelity 3.4- and 13-fold, respectively; in contrast, the Y115A substitution increased the mutation rate fourfold. Only two substitutions in the minor groove-binding tract, G262A and W266A, decreased the fidelity of DNA synthesis by three- to fourfold.

Misinsertion and mismatch extension assays have implicated the primer grip region of HIV-1 RT as being an important determinant of RT fidelity (107). Gutierrez-Rivas and Menendez-Arias made an interesting observation that the M230I primer grip mutation increased the rate of T-G misinsertions 16-fold, and prolonged passage of a virus containing this mutation resulted in the outgrowth of a revertant that possessed the M230I and Y115W mutations (108). The double mutant had a nearly wild-type efficiency of T-G misinsertions. This result indicated that the primer grip residue M230 and the dNTP-binding-site residue Y115 interacted with each other and the misinsertion defect of the M230I mutation was restored by the Y115W dNTPbinding-site mutation.

These in vitro studies have identified the fingers domain, the primer grip, and the minor groove-binding tract (alpha helix H) region of the thumb domain as important determinants of in vitro fidelity. In general, mutations in the fingers domain (F61, K65, L74, D76, and R78) appear to decrease the *in vitro* forward mutation rate; these results suggest that the wild-type residues at these positions in the fingers domain decrease the accuracy of DNA synthesis. Interestingly, the K65 residue in the fingers domain contacts the triphosphate moiety of the dNTP substrate and the K65R substitution increased the *in vitro* fidelity by eightfold. This result suggests that the K65R substitution increases nucleotide selectivity. Similarly, the Q151 residue contacts the base of the dNTP substrate and the Q151N substitution also increases the in vitro fidelity 13fold.

Mutations of alpha helix H residues G262 and W266 that contact the template-primer in the minor groove

increase the mutation rate (97, 98). Preliminary studies of mutations introduced at similar positions in MLV RT suggest that they also increase the mutation rate *in vivo* (Svarovskaia and Pathak, unpublished results). The alpha helix H of the thumb domain has been proposed to be an important component of a "helix clamp" that maintains contact with the template-primer complex during the translocation step of polymerization (109, 110). The G262 and W266 residues make sequence-independent contacts with the DNA primer 2 to 6 nt upstream of the 3' end of the primer (98, 111). These mutations were shown to decrease template affinity, processivity, frameshift fidelity, and the total amount of full-length DNA product generated.

## 8.4. The role of MLV RNase H primer grip and template-primer structure in fidelity

Recent analysis of the MLV RT Y586F mutant has provided novel insights into the structural features of the reverse transcription complex that are important for accuracy of DNA synthesis (104). The Y586 residue of MLV RT is part of a conserved DSXY motif that is present in most retroviral RNase H domains as well as *E. coli* RNase H. The MLV RT Y586 residue is equivalent to the HIV-1 Y501 residue, which is a component of the recently identified RNase H primer grip domain (99). One function of the RNase H primer grip domain and the Y501 residue, which contacts the DNA primer strand, is to position the template-primer near the RNase H active site and control RNase H cleavage specificity (112).

Zhang and colleagues determined the effect of the MLV Y586F mutation on the *in vivo* forward mutation rate. The presence of the Y586F substitution was associated with a 5.4-fold and a 4.3-fold increase in the forward mutation rates of the *lacZ* and GFP reporter genes, respectively. A summary of the characterization of the mutations induced in the GFP gene is shown in table 5. The results indicated that the frequency of substitution mutations increased approximately sixfold while the frequencies of frameshift mutations and other template switching mutations also increased about threefold.

Further analysis of the substitution mutations indicated that a large proportion of the substitutions induced by the Y586F mutation were clustered near adenine-thymine tracts (AAAA, TTTT, and AATT), which are known to induce bends in DNA (figure 4). The adenine-thymine tracts, also referred to as A-tracts, were present within 18-nt of 81% of the substitutions induced by the Y586F mutation. The high proportion of substitutions at these sites represented a 17.2-fold increase for substitutions near A-tracts in comparison to the wild-type RT (table 5).

These results indicated that the Y586F mutant is a mutator RT. What is the possible explanation for the strong correlation between the Y586F mutation and the increase in substitutions within 18 nt of A-tracts? Because the A-tract sequences are associated with bends in DNA, the

|--|

BYD         Factor         Factor <th>Mutation</th> <th>Description</th> <th>Assay – Reporter gene</th> <th>Relative mutant frequency</th> <th>Reference</th>	Mutation	Description	Assay – Reporter gene	Relative mutant frequency	Reference
Inde Data Procession International Loss         International Procession Proce	HIV-1			Les my	
No.9         Page 1         Page 2         Page 2 </td <td>F61A</td> <td>Fingers</td> <td>In vitro FMA – lacZa</td> <td>↓11.7X</td> <td>121</td>	F61A	Fingers	In vitro FMA – lacZa	↓11.7X	121
Party	K65R	Fingers/dNTP binding/ddf	In vitro FMA – lacZa	↓8.1 X	122
LAY     Ingrade     A second A, and A, and A     Lat X     Lat X       DW     Norm     A second A, and A     Lat X     Descender       BCA     Prom     A second A, and A     Lat X     Descender       BCA     Prom     A second A, and A     Lat X     Descender       BCA     Prom     A second A, and A     Lat X     Descender       Main and A     Lat X     Descender     Descender     Descender       Main and A     Lat X     Descender     Descender     Descender       Main and A     Lat X     Descender     Descender     Descender       Main and X     Lat X     Descender     Descender     Descender       Main A     Main A     Descender     Descender     Descender       Main A     Main A     Descender     Descender     Descender       Y LIS     A     Descender     Desce	R72A	Fingers	In vitro FMA – lacZa	11.6X	123
Diff         Paper         Paper March 1, 15 Yes         Diff           Filter         Paper March 1, 15 Yes         Diff         Diff         Diff           Filter         Paper March 1, 15 Yes         Diff         Diff         Diff           Filter         Paper March 1, 15 Yes         Diff         Diff         Diff           Filter         Paper March 1, 15 Yes         Diff         Diff         Diff           Filter         An UP March 1, 15 Yes         Diff         Diff         Diff           Filter         An UP March 1, 15 Yes         Diff         Diff         Diff           Filter         An UP March 1, 15 Yes         Diff         Diff         Diff           Filter         An UP March 1, 15 Yes         Diff         Diff         Diff           Filter         An UP March 1, 15 Yes         Diff         Diff         Diff           Filter         An UP March 1, 15 Yes         Diff         Diff         Diff           Filter         An UP March 1, 15 Yes         Diff         Diff         Diff           Filter         An UP March 1, 15 Yes         Diff         Diff         Diff           Filter         An UP March 1, 15 Yes         Diff         Diff         Diff     <	L74V	Fingers/ddf	In vitroFMA – lacZa	↓ 1.7 X	122
DNA         Figns         Autor MA are between the second s			In vitroFMA – lacZa	↓ 3.5X	124
Parts         Mature is a second	D76V	Fingers	In vitro FMA – lacZa	↓8.8X	125
BAA BOA BOA BOA BOA BOA BOA BOA BOA BOA			Misinsertion	↓10X (A-C)	
BSG         Pages/Source <sup>1</sup> Jurnal Max-lock         List Normal Mathematics         List Normal Mathematics         List Normal Mathematics         List Normal Mathematics         List Normal Mathematics           PRGP         Pages/TC <sup>4</sup> Amound Max-lock         List Normal Max-lock         Dist           NUES         Amound Max-lock         List Normal Max-lock         Dist           VIIA         Art Printing         Manage Mathematics         Dist           VIIA         Art Printing         Manage Mathematics         Dist           VIIA         Art Printing         Manage Mathematics         Dist           VIIA         Art Printing         Manage Antonion         Dist         Dist           VIIA         Art Printing         Manage Antonion         Dist         Dist         Dist           VIIA         Art Prin	R78A	Fingers	In vitro FMA – lacZa	↓8.9X	126
Process of the second secon	E89G	Fingers/foscarnet <sup>R</sup>	In vitro FMA – lacZa	↓1.4X	127
Link of the second state is a second state second state is a second state is a second state is a			Mismatch extension	10X (G-A)	128
Proc.         Proc.         Ministrike A strain           Y 115         A Strain         A strain         Ministrike A strain			In vitro FMA – lacZa	↓2X	124
BRGC MARCE         Progent C         Advance Advance C         11 X (G)         P2           Y115A         APTPreduct         Advance Advance C         13 X (GA)         13           Y115A         APTPreduct         Advance Advance C         14 X (GA)         13           Y115A         APTPreduct         Manue Advance C         14 X (GA)         13           Y115Y         APTPreduct         Manue Advance C         14 X (GA)         13           Y115Y         APTPreduct         Manue Advance C         13 X (X, GA)         10           Y115Y         APTPreduct         Manue Advance C         13 X (X, GA)         10           Y115Y         APTPreduct         Manue Advance C         13 X (X, GA)         10           Y115Y         APTPreduct         Manue Advance C         13 X (X, GA)         10           Y115Y         APTPreduct         Manue Advance C         13 X (X, GA)         10           Y115Y         APTPreduct         Manue Advance C         13 X (X, GA)         10           Y115Y         APTPreduct         Manue Advance C         13 X (X, GA)         10           Y115Y         APTPreduct         Manue Advance C         14 X (X, GA)         10           Y115Y         APTPreduct			Misinsertion	↓16.8X (T-G)	129
Minor         Minoria exclusion         1XX G-D PA         B           Y115A         APTPhalag         A nord/MA-Les/2         100           Y115Y         APTPhalag         Minoria exclusion         12X G-D TKK A-D TKK	E89G/	Fingers/3TC <sup>R</sup>	In vitroFMA – lacZa	↑ 1.4 X	127
Manufa canada         I.N. (Cr.)         PI           YI12A         APT Handag         Interview         Interview         Interview           YI12A         APT Handag         Interview         Interview         Interview           YI12A         APT Handag         Interview         Interview         Interview         Interview           YI12A         APT Handag         Manufa canada         Interview         Interview         Interview         Interview           YI12A         APT Handag         Manufa canada         Interview	M184V				
Y115A         Ar UPTMaining         Jate and the second of			Mismatch extension	↓ 1.3X (G-G)	128
Y15A         Altribuiling         A start/MAlar.Z         Y K         154           Minus de control         Y KO (-O)         30           Y115V         ADTPinning         Minus de control         1 KX (-O)         30           Y115V         ADTPinning         Minus de control         1 KX (-O)         30           Y115V         ADTPinning         Minus de control         1 KX (-O)         30           Y1151         ADTPinning         Minus de control         7 KX (-O)         30           Y1151         ADTPinning         Minus de control         7 KX (-O)         30           Y1151         ADTPinning         Minus de control         7 KX (-O)         30           Y1151         ADTPinning         Minus de control         7 KX (-O)         30           Y1151         ADTPinning         Minus de control         7 KX (-O)         30           Y1152         ADTPinning         Minus de control         7 KX (-O)         30           Y1154         ADTPinning         Minus de control         7 KX (-O)         30           Y1157         ADTPinning         Minus de control         7 KX (-O)         30           Y1158         ADTPinning         Minus de control         7 KX (-O)         30				↑29X (G-A)	
Mainerine         Mainerine         Mainerine         Mainerine         Mainerine         Mainerine         Mainerine           V115Y         APTPendag         Mainerine         1 AX AC O         13           Y115         APTPendag         Mainerine         1 AX AC O         131           Y115         APTPendag         Mainerine         1 XX AC O         131           Y115         APTPendag         Mainerine         1 XX AC O         131           Y115         APTPendag         Mainerine         1 XX AC O         131           Y115         APTPendag         Mainerine destration         1 XX AC O         131           Y115         APTPendag         Mainerine destration         1 XX AC O         131           Y115         APTPendag         Mainerine destration         1 XX AC O         131           Y115         APTPendag         Mainerine destration         1 XX AC O         131           Y115         APTPendag         Mainerine destration         1 XX AC O         131           Y115         APTPendag         Mainerine destration         1 XX AC O         131           Y115         APTPendag         Mainerine destration         1 XX AC O         131           Y115	Y115A	dNTPbinding	In vitro FMA – lacZa	↑4X	124
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Member density         Luk A-O         11           Y115Y         AVTPhalag         12         14           W114L         AVTPhalag         12         12           Y114L         AVTPhalag         Member density         73         12         12           Y115L         AVTPhalag         Member density         73         12         12           Y115L         AVTPhalag         Member density         73         13         12           Y115L         AVTPhalag         Member density         74         12         12           Y115L         AVTPhalag         Member density         74         12         12           Y115L         AVTPhalag         Member density         74         12         12           Y115L         AVTPhalag         Member density         12         12         12         12         12         <				↑ 10X (A-G)	
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Memade dension1 4.6X (A.C)13YI B.APPendangMemade densionT15X (A.C)13YI D.CAPPendangMemade densionT15X (A.C)13YI D.CAPPendangMemade densionT15X (A.C)13YI D.CAPPendangMemade densionT15X (A.C)13YI D.CAPPendang densionT25X (A.C)1314YI D.SAPPendang densionT25X (A.C)1415YI D.SAPPEndang densionT15X (A.C)1314YI D.SAPPEndang densionT15X (A.C)1415YI D.SAPPEndang densionT15X (A.C)1415YI D.SAPPEndang densionT15X (A.C)1515YI D.SAPPEndang densionT15X (A.C)1515YI D.SAPPEndang densionT15X (A.C)1515YI D.SAPPEndang de			Misinsertion	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	130
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Yilal.     dbTPinding     Manake densime     Tab.S.A.G.O.     Bil       Yilal.     dbTPinding     Manake densime     Tab.S.A.G.O.     Bil       Yilas     dbTPinding     Manake densime     Tab.S.A.G.O.     Bil       Qistim     dbTPinding     Manake densime     Tab.S.G.O.			Mismatch extension	↑ 3125X (A-G)	130
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V1181 V1181 V1181 V1181 V1181eNTPlacing V1182 eNTPlacing OPTPlacing V1182Meanual accession V1182 Namenal accession V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1182 V1184 V11	Y115I	dNTP binding	Mismatch extension	13.8X (A-C)	131
V115M V115M V115Gof NTPaining NUMBAR centron NUMBAR centron NUM	Y115N	dNTP binding	Mismatch extension	↑ 17.8X (A-C)	131
Y1151dNTPindingMenucle cleanesisTALK (A.C)13Y1152dNTPindingMenucle cleanesisTALK (A.C)13Y1152dNTPindingMenucle cleanesisTALK (A.C)13Y1154dNTPindingMenucle cleanesisTALK (A.C)13Y1155dNTPindingMenucle cleanesisTALK (A.C)13Y1155dNTPindingMenucle cleanesisTALK (A.C)13Y1157dNTPindingMenucle cleanesisTALK (A.C)13Q151MdNTPindingMenucle cleanesisTALK (A.C)13Q151MdNTPindingMenucle cleanesis14.5 X C.A)13Q151MdNTPindingMenucle cleanesis1.1 X13Y176MatelengA stra-PMA - Leane1.1 X13Y177MatelengMenucle cleanesis1.1 X13Y178MatelengA stra-PMA - Leane1.1 X13Y179MatelengMenucle cleanesis1.1 X13Y187PalanMenucle cleanesis1.1 X13Y187Y187Y180Menucle cleanesis1.1 X13Y187Y180Menucle cleanesis1.1 X1313Y187Y180Menucle cleanesis1.1 X1413M1841YMDDMenucle cleanesis1.1 X1313M1841YMDDMenucle cleanesis1.1 X1414M1841YMDDMenucle cleanesis1.1 X1414M1841YMDD	Y115M	dNTP binding	Mismatch extension	↑ 3X (A-C)	131
Y13GAPTPindingMomale densition manuel densition manuel densition to DSX (AC)13 10 SX (AC	Y115H	dNTP binding	Mismatch extension	↑ 8.1X (A-C)	131
V15CdNP1shingManual contentsT 4 M (AC)11V15CdNP1shingMounch contentsT 10.55 (AC)13V15SdNP1shingMounch contentsT 25.55 (AC)13V15SdNP1shingMounch contentsT 25.55 (AC)13V15SdNP1shingMounch contentsT 25.55 (AC)13V15WdNP1shingMounch contentsT 25.55 (AC)13V15WdNP1shingMounch contentsT 25.55 (AC)13V15WdNP1shingMounch contentsT 25.55 (AC)13V15WdNP1shingMounch contentsT 25.55 (AC)13Q15MdNP1shingMounch contentsT 25.55 (AC)13AVVMail deg*te viroPMA-facZ13.55 (AC)13V15WdNP1shingMounch contentsT 15.52 (AC)13P100PalmMounch contentsT 15.52 (AC)13P100PalmMounch contentsT 15.52 (AC)13P100PalmMounch contentsT 15.52 (AC)13P100PalmMounch contentsT 15.52 (AC)13M184VYMD0.700Mounch contentsT 15.52 (AC)13M184VYMD0.700Mounch contentsT 15.52 (AC)13M184PYMD0.700Mounch contentsT 15.52 (AC)13M184PYMD0.700Mounch contentsT 15.52 (AC)13M184PYMD0.700Mounch contentsT 15.52 (AC)13M184PYMD0.700Mounch c					
Miles Miles Miles Miles MilesMeand cension Meand cension<	Y115G	dNTP binding	Mismatch extension	T 34.9X (A-C)	131
N1SC N1SC N1SCAMP inding AMP inding AMP inding Manafi cettomio PAX ACD110 (ACD) TAX ACD131N1SC N1SFATP inding Markat cettomio Manafi cettomio PAX ACD12X (ACD)131N1SWATP inding Markat cettomio Manafi cettomio PAX ACD13X (ACD)138Q1SIMATP inding Mail and Markat cettomio PAX13X (ACD)131Q1SIMATP inding Mail and Markat cettomio PAX13X (ACD)131Q1SIMATP inding Mail and Markat cettomio PAX14X (ACD)131Q1SIMATP inding Mail and Markat cettomio PAX14X (ACD)131Q1SIMATP inding Mail and Q1SIM14X (ACD)135Q1SIMATP inding Mail and Markat cettomio PAR14X (ACD)135P100PalmMarkat cettomio Mail and Markat cettomio PAR11X (ACD)135P100PalmMarkat cettomio Mail and Markat cettomio PAR12X (ACD)135P100PalmMarkat cettomio Mail and Markat cettomio11X (ACD)136P100Markat cettomio Mail and Markat cettomio12X (ACD)135P100PalmMarkat cettomio Mail and Markat cettomio12X (ACD)135P101PalmMarkat cettomio Mail and Markat cettomio12X (ACD)136P102PalmMarkat cettomio Mail and Markat cettomio12X (ACD)136M104PalmMarkat cettomio Mail and Markat cettomio12X (ACD)136<			Mismatch extension	T 5416X (A-G)	130
Y155     MPT binding Abacoir     Meanste Creasion     7253 (AC)     131       Y1157     APT Pinding Abacoir     In trov PAA - dz2a     14 fs X     16 fs X       Y1158     APT Pinding Abacoir     In trov PAA - dz2a     14 fs X     16 fs X       Y1159     APT Pinding Muid-off     If wire PAA - dz2a     14 fs X     15 fs Is       AVV     Ministerior     PAA - dz2a     13 X     15 fs Is       AVV     Ministerior     PAA - dz2a     13 X     15 fs Is       AVV     Ministerior     PAA - dz2a     13 X     15 fs Is       AVV     Ministerior     PAA - dz2a     13 X     15 fs Is       Y159     APT Pinding     Ministerior     PAA - dz2a     13 X     15 fs Is       Y159     PAI     Ministerior     PAA - dz2a     13 X     15 fs Is       Y159     PAI     Ministerior     13 K     15 fs Is     15 fs Is       Y159     PAI     Ministerior     13 K     15 fs Is     15 fs Is       Y159     PAI     Ministerior     13 K A (AC)     13 fs Is     15 fs Is       Y159     PAI     Ministerior     13 K (AC)     13 fs Is     15 fs Is       Y159     Y150     PAI     Ministerior     13 K (AC)     13 fs Is	Y115C	dNTP binding	Mismatch extension	↑ 10.5X (A-C)	131
Y115drTPinningbrimPTMA-lacZa1 J K7474V115WdrTPinningMinasetia< consoina	Y115S	dNTP binding	Mismatch extension	↑25.3X (A-C)	131
V15F         dvTPinding / Abasivi         h viroPMA-lar.2z         1 L5 X         74           V15W         dVTPinding         Mismath extension         72.0X (AC)         103           V15W         dVTPinding / Multi-dong?         Mismath extension         73.0X (AC)         108           Q151M         dVTPinding / Multi-dong?         Mismath extension         14.0X (AC)         105.123           Q151M         dVTPinding / Multi-dong?         Mismath extension         11.0X         105.123           Q151N         dVTPinding / Multi-dong?         Mismethine - RNA         14.0X (CA)         105.133           Q151N         dVTPinding / Multi-dong?         PriroPMA-lar.2z         1.1X (CA)         105.133           Q151N         dVTPinding / Multi-dong?         PriroPMA-lar.2z         1.1X (CA)         135           Q151N         dVTPinding / Multi-dong?         PriroPMA-lar.2z         1.1X (CA)         135           Q151N         dVTPinding / Multi-dong?         Missortion         1.1X (CA)         135           Q151N         dVTPinding / Multi-dong?         Missortion         1.1X (CA)         135           Q151N         dVTPinding / Multi-dong?         Missortion         1.1X (CA)         135           Q151N         dVTPinding / Multi-dong?					
VILW VILW OUSIMAVTPbinding Multi-dreg"Missaction Missaction Multi-dreg"Liss (A-C) Missaction SAUX (A-C)131 BAUX (A-C)132 BAUX (A-C)132 BAUX (A-C)132 BAUX (A-C)131 BAUX (A-C)131<	Y115F	dNTP binding/ Abacavir	In vitro FMA – lacZa	↓ 1.6 X	74
VILSWof NTPinding Main drg"Missuetion model Missuetion			Mismatch extension	12.6X (A-C)	131
11.0%         ab Printing         Mismethic         1.2 (A2)         100           Q151M         dVTPinding/Micharg/         historiton         1.2 (A2)         103           Q151M         dVTPinding/Micharg/         historiton         1.2 (A2)         103           ACV         Multi-ding/*         historiton         1.2 (A2)         103           ACV         Multi-ding/*         historiton         1.2 (A2)         103           YTPU         T         total (A2)         1.3 (A2)         103           Q151M         dVTPinding         historiton         1.1 (A2)         13           Q151M         dVTPinding         historiton         1.1 (A2)         13           Q151M         dVTPinding         Missoriton         1.1 (A2)         13           Q151M         dVTPinding         Missoriton         1.1 (A2)         13           Q151M         dVTDD         Missoriton         1.1 (A2)         13           Y18F         VADD         Missoriton         1.1 (A2)         13           M184V         VADD         Missoriton         1.1 (A2)         13           M184V         VADD         Missoriton         1.1 (A2)         13           Missoriton	N1150	DIMENT I	NO	1a 57 (1 6)	100
QU51M         drTP binding/Multi-drug <sup>2</sup> Ministration mathematics         14.5X         CAC         152           ACV         Mail ording <sup>4</sup> In vitroPMA-lacZa         1.15X         105         105           ACV         Mail ording <sup>4</sup> In vitroPMA-lacZa         1.15X         105         105           V13P         Fillow         In vitroPMA-lacZa         1.15X         105         105           Q151M         dNTPbinding         BrinzefMA-lacZa         113 N         105,13         105           Q151M         dNTPbinding         BrinzefMA-lacZa         113 N         105,13         105           Q151M         dNTPbinding         BrinzefMA-lacZa         113 N         105,13         105           Q151A         Ministerition         T1,13 N, N          105,13         105         105           Q151A         Palin         Misserition         T1,13 N, N          105         105         105           Y183F         YMDD         Misserition         T1,14 N, N          104         104         104         104         104         105         104         104         104         105         104         105         104         104         104         104         104	¥115W	dNTPbinding	Misinsertion	13.5X (A-C)	108
Op/SM         Mit Printing Multi-ang         Mit month A - Jac 2         1, 2X, C, A)         10           AGX'         Mit elong a         Mit month A - Jac 2         1, 1X         13           AGX'         Mit elong a         Mit month A - Jac 2         1, 1X         13           YIN         TTU         TY         13X         105           YIN         Mitsention - RNA         LXX         105           Q151M         MTTU         13X         105           Q151M         Mitsention - RNA         LXX         12           Q151M         Mitsention - RNA         LXX         14           PIGW         Pain         Mitsention - RNA         LXX         15           PIGW         Pain         Mitsention - RNA         LXX         16           YIEF         YMDD         Mitsention - RNA         LXX         16           MISAV         YMDD/TC*         Mitsention - RNA         LXX         16           MISAV         YMDD/TC*         Mitsention - RNA         LXX         16           MISAV         YMDD/TC*         Mitsention - RNA         LXX         17           MISAV         YMDD/TC*         Mitsention - RNA         LXX         16	015114	DEDUCT OF STATE	Mismatch extension	8.0X (A-C)	131
Auth-drog <sup>a</sup> Main-drog <sup>b</sup> L         L <thl< th=""> <thl< th=""> <thl< th="">         L</thl<></thl<></thl<>	QISIM	dN I P binding/ Multi-drug	In vitro FMA – lacZa	↓1.2X	105, 132
Mail         Mail drog <sup>2</sup> In stropPAL-lacZa         J. J. X         J.2           FT/L FT/L FT/L FT/L FT/L FT/L FT/L OUSIN         dNTPbinding         In stropPAL-lacZa         LJ.X         105.133           QUSIN         dNTPbinding         In stropPAL-lacZa         LJ.X         14           QUSIN         dNissertion         T.1.X         15           FIGO         Palm         Missertion         T.1.X         16           FIGON         Palm         Missertion         T.1.X         13           YIRSF         Palm         Missertion         T.1.X         13           Missertion         LLX (AC)         135         13           YIRSF         Palm         Missertion         LLX (AC)         135           MISAV         YMDD         In stropPAL-lacZa         LLX (AC)         136           MISAV         YMDD         In stropPAL-lacZa         LLX (AC)         137           MISAV         YMDD         In stropPAL-lacZa         LLX (AC)         138           MISAU         YMDD         Missertion         LLX (AC)         137           MISAU         YMDD         Missertion         LLX (AC)         138           MISAU         YMDD         Mis		B	Misinsertion – KNA	↓ 6.5 X (C-A)	
Valimeter         Valimeter         413 X         105 133           Q151M         Q151M         dX1 b los 0.5 X         13           Q151M         Q151M         13 X         13 X           Q151M         Plan         13 X         13           Misserbina - RoZa         1, 11 X         14 X         13           Plan         Misserbina         1, 11 X         14 X         13           N184P         Palan         Misserbina         1, 11 X         14 X         13           N184P         Palan         Misserbina         1, 11 X         14 X         13           N184P         YMDD         In wirroPMA - lacZa         1, 16 X         13         13           Misserbina         1, 12 X         13         13         13         13           Misserbina         1, 11 X         14 X         13         13         13           Misserbina         1, 11 X         14 X         13         13         13           Misserbina         1, 11 X         14 X         13         13         13           Misserbina         1, 13 X         13 X         13         13         13           Misserbina         1, 20 X         1, 20	A62V/	Multi-drug <sup>*</sup>	In vitroFMA – lacZa	↓ 1.7 X	132
F717/F         F134         05.13           Q151N         dVTPinding         In vinoPMA - IncZa         13 X         05.13           K154A         In vinoPMA - IncZa         12.1X         134           F160V         Palm         Misnicetica         12.1X         135           F160V         Palm         Misnicetica         12.1X         135           F160N         Palm         Misnicetica         12.1X         135           Y18F         YMDD         Misnicetica         11.1X         14.6X           Misnicetica         11.1X         135         135           M184V         YMDD         Misnicetica         11.1X         135           Misnicetica         11.2X         136         137           Misnicetica         11.2X         137         138           M184V         YMDD/TC*         Hirtfor/MA - IncZa         11.2X         137           Misnicetica         13.2X (G7)         139         137           Misnicetica         14.2X (C1)         142         143           M184         YMDD         Misnicetica         140         141           M184         YMDD         Misnicetica         140         141 <t< td=""><td>V/51/</td><td></td><td></td><td></td><td></td></t<>	V/51/				
Flore QUSIN         dVTPinning         humoPMA - IndZa         J3 X         05.133           K154A         HumoPMA - IndZa         J2 X         134           FlorOV         Palm         Missinetrion         1.9.21 X         135           FlorOV         Palm         Missinetrion         1.9.21 X         135           FlorOV         Palm         Missinetrion         1.1 X (AcO)         135           FlorOV         Palm         Missinetrion         1.2 X (AcO)         135           Y183F         YMDO         In winoPAA - IndZa         1.6 X         126           Missinetrion         1.3 - 4.9 X         136         137         136           Missinetrion         1.3 - 4.9 X         136         137         136           Missinetrion         1.3 - 4.9 X         137         137         136           Missinetrion         1.3 X (GT)         128         137         136           Missinetrion         1.3 X (GT)         138         136         137           Missinetrion         1.3 X (GT)         136         137         136           Missinetrion         1.3 X (GT)         136         137         136           Missinetrion         1.3 X (GT)	F77L/				
Q121A Q151N Q151Ndr.TPPinding <i>in virroPMA -lacZa</i> <i>lar virroPMA -lacZa</i> L1X LX (b2,5X)U5,13K134A F160YPalmMismetrion1,19-2,1X13F160WPalmMismetrion1,11X (AC)135F160WPalmMismetrion1,11X (AC)13Y187FYMDDMagae Education1,11X (AC)136M184VYMDDMismetrion1,11X (AC)136M184VYMDDMismetrion1,12X136M184VYMDDSTC*In virroPMA-lacZa1,12X136M184VYMDDSTC*In virroPMA-lacZa1,12X137M184LYMDDSTC*In virroPMA-lacZa1,12X137M184LYMDDTC*In virroPMA-lacZa1,15X (GT)139M184LYMDDMismetrion1,15X (GT)139M184LYMDDMismetrion1,15X (GT)130M184LYMDDMismetrion1,15X (GT)140M184AYMDDMismetrion1,15X (GT)140M184AYMDDMismetrion1,15X (GT)140M184AYMDDMismetrion1,15X (GT)140M184AYMDDMismetrion1,15X (GT)165P22APirme gripMismetrion1,15X (GT)165M184AYMDDMismetrion1,15X (GT)165M184AYMDDMismetrion1,15X (GT)165M184AYMDDMismetrion1,15X (GT)165M184AYMDD<	F116Y/				
Q DNADM PRIMARYDA Loc 20DA Loc 20K154AIn the Prince of Control	QISIM	ANTTOIN : - dia -	L TMA L	12 8	105 122
K15A     Alignment KAA     43.X     K3A     14       F160Y     Pain     Mismach cension     11.X     (A)     15       F160W     Pain     Mismach cension     11.X     (A)     15       F160W     Pain     Mismach cension     11.X     (A)     15       Y18F     YMDD     Mismach cension     12.X     (A)     15       Mismach cension     11.1     (A)     12     (A)     15       Mismach cension     11.X     (A)     15     (A)     15       Y18F     YMDD     Mismach cension     13.X     15     (A)     12       Mismach cension     13.X     13.X     13     (A)     (A)     (A)       Mismach cension     13.X     13.X     13     (A)     (A)     (A)       Mismach cension     13.X     (A)     13     (A)     (A)     (A)     (A)       Mismach cension     13.X     (A)     13     (A)	QISIN	dis i P binding	IN VIITO FMA – IACZA		105, 155
N.54         Palm         Interprise         1.1         1.5         1.5           F160         Palm         Misimetrion         17.5         1.5         1.5           F160         Palm         Misimetrion         12.1X         1.6         1.5           Y183F         YMDD         In simetrion         1.1         1.4         1.35           Y183F         YMDD         In simetrion         1.1         1.4         1.36           M184V         YMDD3TC <sup>10</sup> In simetrion         1.3X         1.35           M184V         YMDD3TC <sup>10</sup> In simetrion         1.3X         1.35           M184V         YMDD3TC <sup>10</sup> In simetrion         1.3X         1.35           M184         YMDD3TC <sup>10</sup> In simetrion         1.3X         1.35           M184         YMDD3TC <sup>10</sup> In simetrion         1.4X         1.4X           M184         YMDD3TC <sup>10</sup> Misimetrion         1.4X         1.4X           M184         YMDD         Misimetrion         1.4X         1.4X           M184         YMDD         Misimetrion         1.4X         1.4X           M184         YMDD         Misimetrion         1.4X         1.4X <td>W154A</td> <td></td> <td>MISINSETION – RINA</td> <td>↓8.3X to ↓26.5X</td> <td>124</td>	W154A		MISINSETION – RINA	↓8.3X to ↓26.5X	124
Pio0 <sup>1</sup> Pialm     Missiertion     11.9 - 2.1X     135       PiGW     Pialm     Missiertion     11.5 K (A-C)     135       Y183F     YMDD     Mispir Extension     12.6X (A-C)     135       Y183F     YMDD     Misriperfloat     11.6X     124       Missiertion     4.1.1 - 1.4X     136       Missiertion     4.1.4 - 4.9X     136       Missiertion     4.1.4 - 4.9X     136       Missiertion     1.1.5X     124       Missiertion     1.1.5X     127       Missiertion     1.1.5X (G-T)     137       Missiertion     1.1.5X (G-T)     138       Missiertion     1.1.5X (G-T)     137       Missiertion     1.1.5X (G-T)     137       Missiertion     1.1.5X (A-C)     137       Missiertion     1.1.5X (A-C)     137       Missiertion     1.1.5X (A-C)     136       Missieretion     1.1.1.1X (A-C)     136   <	K154A F1COV	D-1	In VIITO FMA – IACZA	↓ 2.1X	134
FIGWPalmMismacrion11 LV (AC)15FIGWPalmMismacrion12 K(AC)135Y183FYMDDh stror PMA -lacZa1 LX136Y183FYMDDMismacrion1 J 1 - 1 4 X136Mismacrion1 J 1 - 1 4 X136136Mismacrion1 J 1 - 1 4 X136136Mismacrion1 J 2 X136137Mismacrion1 J 2 X (G)137138Mismacrion1 J 2 X (G)140142, 143Mismacrion1 J 2 X (G)140141Mismacrion1 J 2 X (G)140141Mismacrion1 J 2 X (G)140141Mismacrion1 J 2 X (G)160160Mismacrion1 J 2	F160 Y	Palm	Misinsertion	11.9-2.1X	135
FieldwPainMissertion(21K (AC)15Y18FYMDD <i>hr viroPAA-lacZa</i> 7, 6X133Y18FYMDD <i>hr viroPAA-lacZa</i> 7, 6X136M184VYMDDTC* <i>hr viroPAA-lacZa</i> 1, 12X136M184VYMDDTC* <i>hr viroPAA-lacZa</i> 1, 12X124M184VYMDDTC* <i>hr viroPAA-lacZa</i> 1, 12X124M184VYMDDTC* <i>hr viroPAA-lacZa</i> 1, 12X124M184VYMDDTC* <i>hr viroPAA-lacZa</i> 1, 12X128M184IYMDDTC* <i>hr viroPAA-lacZa</i> 1, 12X128M184IYMDDTC* <i>hr viroPAA-lacZa</i> 4, 40X4, 21, 43M184IYMDDTC* <i>hr viroPAA-lacZa</i> 4, 40X4, 21, 43M184AYMDDMissertion -RNA4, 40X107M184AYMDDMissertion -RNA1, 12X (C)136W184YMDDMissertion - NA1, 12X (C)136W184YMDDMissertion - NA1, 12X (C)136W184YMDDMissertion1, 12X (AC)136W230APinor grpMissertion1, 5X (A)107M200Pinor grpMissertion1, 12X (A)107M201Pinor grpMissertion1, 12X (A)107M202Pinor grpMissertion1, 12X (A)107M203Pinor grpMissertion1, 12X (A)107M204Pinor grpMissertion1, 12X (A)104M205Pinor grp <t< td=""><td></td><td></td><td>Mismatch extension</td><td>11.1X (A-C)</td><td>135</td></t<>			Mismatch extension	11.1X (A-C)	135
Y13F         YMDD         Major Eduction         12.6X (AC)         15           Misserion         4 1.1 - 1.4X         136           Misserion         4 1.1 - 1.4X         136           M184V         YMDDSTC*         h wirePMA-lac2a         1.1X         137           Misserion         1.1X         137         137           Misserion         1.1X         137         137           Misserion         1.1X         138         137           Misserion         1.2X (C7)         138         138           Misserion         4.3X (C7)         138         138           Misserion         7.4X (C7)         142         142           Misserion         7.4X (C7)         142         143           M184         YMDD         Misserion         7.4X (C7)         160           M184         YMDD         Misserion         7.4X (C7)         160           W22A         YMDD         Misserion         1.5X (AC)         16           W22A         YMDD         Misserion         1.5X (AC)         16           W22A         YMDD         Misserion         1.5X (AC)         16           W22A         YMDD         Misserion	F160W	Palm	Misinsertion	12.1X (A-C)	135
Y187       YMD       h wirePMA-lacZa       TLoX       124         Misnertion $4, 1, -1, 4X$ 136         M184V       YMDD/STC *       Misnertion $4, 1, -1, 4X$ 136         M184V       YMDD/STC *       Misnertion $4, 34, -4, 9X$ 137         M184V       YMDD/STC *       Misnertion $1, 3X$ 137         Misnertion - RNA $45X$ (C7)       138         Misnertion - RNA $45X$ (C7)       139         M184I       YMDD/STC *       Misnertion - RNA $45X$ (C7)       139         M184I       YMDD       Misnertion - RNA $40X$ (C7)       130         M184L       YMDD       Misnertion - RNA $41X$ (C7)       130         M184L       YMDD       Misnertion - RNA $41X$ (C7)       130         M184L       YMDD       Misnertion       110, 10, C7       130         M184L       YMDD       Misnertion       15, X(AA)       107         M184L       YMDD       Misnertion       15, X(AA)       107         M184L       YMDD       Misnertion       15, X(AA)       107         M184L       YMDD       Misnertion       15, X(AA)       107 </td <td></td> <td></td> <td>Mispair Extension</td> <td>12.6X (A-C)</td> <td>135</td>			Mispair Extension	12.6X (A-C)	135
Mississerition         1,1,1,4,X         136           Missuch Extension $3,4,4,9X$ 136           Missuch Extension $3,4,4,9X$ 126           Missuch Extension $1,2X$ 127           Missuch Extension <td< td=""><td>Y183F</td><td>YMDD</td><td>In vitro FMA – lacZa</td><td>11.6X</td><td>124</td></td<>	Y183F	YMDD	In vitro FMA – lacZa	11.6X	124
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			Misinsertion	$\downarrow 1.1 - 1.4X$	136
M184VYMDD STC $^{a}$ In third PMA -lacZa hird PMA -lacZa1.12X124 1.2XM184VVMDD STC $^{b}$ in third PMA -lacZa Misnach Extension RNA Misnach Extension RNA 			Mismatch Extension	↓3.4–4.9X	136
misrack $\downarrow 1.6X$ 127         Misrack       Misrack       15X (GT)       128         Misrack       Misrack       145X (G)       137         Misrack       Misrack       175X (GT)       138         Misrack       Misrack       175X (GT)       138         Misrack       Misrack       1475X (GT)       124         Misrack       Misrack       1475X (GT)       124         Misrack       YMDD/3TC *       141       125X (G)       127         Misrack       YMDD       Misrack       126X (A)       142         Misrack       YMDD       Misrack       126X (A)       140         Misrack       YMDD       Misrack       117X (AC)       136         Misrack       YMDD       Misrack       110X (AC)       136         Misrack       YMDD       Misrack       155X (AA)       107         W239A       Prime grip       Misrack insertion       1,5X       107         W2301       Prime grip       Misrack insertion       1,5X       144       144         W266A       Tumbalpha H       hv vitroFMA -lacZa       14X       144         W266A       Tumbalpha I       hv vitroFMA -lacZa </td <td>M184V</td> <td>YMDD/3TC *</td> <td>In vitroFMA – lacZa</td> <td>1.2X</td> <td>124</td>	M184V	YMDD/3TC *	In vitroFMA – lacZa	1.2X	124
Misuach Extension $7.3 X (G-T)$ 128         Misuach Extension - RNA $4.6 X (A, G)$ 137         Missinserion - RNA $4.6 X (A, G)$ 138         Missinserion - RNA $4.6 X (A, G)$ 139         Missinserion - RNA $4.6 X (A, G)$ 138         Missinserion - RNA $4.0 X$ $4.17 \times (C, T)$ Missinserion - RNA $4.0 X$ 147         Missinserion - RNA $4.0 X$ 147         Missinserion - RNA $4.0 X$ 140         Missinserion $1.6 X (C, T)$ 140         Missinserion $1.6 X (T, C)$ 137         Missinserion $1.6 X (T, C)$ 136         Missinserion $1.6 X (T, G)$ 160         Missinserion $1.5 X (A, A)$ 107         Missinserion $1.5 X (A, A)$ 107         W20A       Primer grip       Missinserion $1.5 X (A, A)$ 107         W20A       Primer grip       Missinserion $1.5 X (A, G)$ 108         W20A       Primer grip       Missinserion $1.5 X (A, G)$ 108         W20A       Binding - evertant $1.5 X (A, G)$ 108			In vitroFMA – lacZa	↓ 1.6X	127
Missach Extension – RNA         48,6X (A-G)         137           Missachion – RNA         48,6X (A-G)         138           Missertion         17,5X (C-T)         139-141           Missertion         13,5X (C)         124,1X (C-T)           Missertion         13,5X (C)         124,1X (C-T)           Missertion – RNA         4,0X         122,143           Missertion – RNA         4,0X (A-G)         137           Missertion – RNA         4,0X (A-G)         137           Missertion – RNA         4,0X (A-G)         136           Missertion – RNA         4,0X (A-G)         137           Missertion – RNA         4,0X (A-G)         136           VE204         Primer grip         Missertion         11,1X (A-C)         136           V229A         Primer grip         Missertion         1,5X         107           M2300         Primer grip         Missertion         1,5X         108           Y15W         Binding - evertant         14X (T-G)         108           Q278A         Thumbialpha H         In vitroFMA - lacZa         No Change         145           Q278A         Thumbialpha I         In vitroFMA - lacZa         No Change         145           Q278A			Mismatch Extension	T 3.5X (G-T)	128
Missection         RNA $45X(A)$ 138           Missection $175X(C^{*})$ $19141$ M1841         YMDD/3TC * $130X(C)$ $121X(C)$ M184A         YMDD         Missection-RNA $40X$ $122143$ M184A         YMDD         Missection-RNA $61X(AC)$ $137$ M184A         YMDD         Missection-RNA $61X(AC)$ $140$ M184L         YMDD         Missection-RNA $61X(AC)$ $140$ M184L         YMDD         Missection-RNA $61X(AC)$ $140$ M184L         YMDD         Missection $14X(C)$ $140$ M184L         YMDD         Missection $11X(AC)$ $107$ V229A         Primer grip         Missection $1.5X$ $107$ W229A         Primer grip         Missection $1.5X$ $107$ W230A         Primer grip         Missection $1.5X$ $145$ W230A         Primer grip         Missection $1.5X$ $145$ W230A         Dumb/alpla I         In virorPAA-lacZa			Mismatch Extension – RNA	48.6X (A-G)	137
$\begin{tabular}{ c c c c c } \label{eq:higher} \begin{tabular}{ c c c c c c c c c c c c c c c c c c c$			Misinsertion – RNA	↓6.5X (-A)	138
M1841         YMDD3TC <sup>8</sup> In vitroFMA-lacZa         4.4.0X         142, 143           M184A         YMDD         Mismetion - RNA         4.2.6X (-A)         142, 143           M184A         YMDD         Mismetion - RNA         6.1X (A-C)         137           M184A         YMDD         Mismetion - RNA         6.1X (A-C)         140           M184L         YMDD         Mismetion - RNA         6.1X (A-C)         140           M184L         YMDD         Mismetion - RNA         6.1X (A-C)         140           M184L         YMDD         Mismetion         1.1X (A-C)         160           V220A         Primer grip         Misinserion         1.5X (A-C)         107           W220A         Primer grip         Misinserion         1.6X (T-G)         108           M230V         Primer grip         Misinserion         1.4X (T-G)         108           W230A         Tunmbalpha H         In virroFMA - lacZa         No Chang			Misinsertion	↓17.5X (C-T)	139-141
M1841         YMDD3TC <sup>k</sup> In vitro FMA - lacZa         14.0X         142.143           Misinsertion = RNA         2.6X (A)				↓ 3.6X (-C)	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$				↓ 2.4X (C-T)	
Mismetin - RNA $J_{2}$ CX (A)M184AYMDDMismatch extension - RNA $J_{11}$ (X (A°C)137M184LYMDDMisinsertion $T_{16X}(T)$ 140M184LYMDDMismatch extension $J_{11}$ IX (A°C)136 $J_{22}$ CX (AG) $J_{22}$ CX (AG)136F227APrimer gripMisinsertion $J_{15}$ SX (AA)107W29APrimer gripMisinsertion $J_{15}$ SX (AA)107W2301Primer gripMisinsertion $J_{15}$ SX (AA)108M2301Primer gripMisinsertion $J_{15}$ X (T-G)108W2304Primer gripMisinsertion $J_{15}$ X (T-G)105W2304Primer gripMisinsertion $J_{15}$ X (T-G)105W2304Primer gripMisinsertion $J_{15}$ X (T-G)105W2305Primer gripMisinsertion $J_{15}$ X (T-G)105W2304Primer gripIn virroPMA -lacZaNoChange145Q262ATumbalpha IIn virroPMA -lacZaNoChange145Q278ATumbalpha IIn virroPMA -lacZaNoChange145 <td>M184I</td> <td>YMDD/3TC <sup>R</sup></td> <td>In vitroFMA – lacZa</td> <td>↓4.0X</td> <td>142, 143</td>	M184I	YMDD/3TC <sup>R</sup>	In vitroFMA – lacZa	↓4.0X	142, 143
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			Misinsertion - RNA	↓2.6X (-A)	
M184AYMDDMismatch extension $1_1 K (\Lambda - C)$ 140M184LYMDDMismatch extension $1_1 LX (\Lambda - C)$ 136M184LYMDDMismetrion $1_2 X (\Lambda - G)$ 136F227APrimer gripMisinsertion $1_5 SX (\Lambda - A)$ 107W229APrimer gripMisinsertion $1_1 SX$ 107W2301Primer grip(MTPMisinsertion $1_1 SX$ 108M2301Primer grip(MTPMisinsertion $1_1 SX (T - G)$ 108W2304Binding-revertantIf a X144144C262AThumbialpha HIn vitro/FMA-lacZa $\uparrow$ 3 X144R277AThumbialpha IIn vitro/FMA-lacZaNo Change145Q278AThumbialpha IIn vitro/FMA-lacZaNo Change145Q278AThumbialpha IIn vitro/FMA-lacZaNo Change145L279AThumbialpha IIn vitro/FMA-lacZaNo Change145C280AThumbialpha IIn vitro/FMA-lacZaNo Change145L281AThumbialpha IIn vitro/FMA-lacZaNo Change145L283AThumbialpha IIn vitro/FMA-lacZaNo Change145G285AThumbialpha IIn vitro/FMA-lacZaNo Change145L74VFingersMismatch extension $\downarrow_1 1 X$ 106E89GFingersMismatch extension $\downarrow_1 1 X$ 106L74VS15YFingersMismatch extension $\downarrow_3 X (\Lambda - C)$ 106L74VS15YFingersMismatch ex			Mismatch extension – RNA	↓6.1X (A-C)	137
M184LYMDDMisnatch extension $\downarrow 11, 1X (A-C)$ $\uparrow 36$ H1.1X (A-C) $\downarrow -32X (A-C)$ $\downarrow -32X (A-G)$ $\downarrow -32X (A-G)$ W220APrimer gripMisnsertion $\uparrow 5, 5X (A-A)$ 107M230IPrimer gripMisnsertion $\downarrow 1, 5X$ 107M230JPrimer gripMisnsertion $\uparrow 16X (T-G)$ 108Y115WBinding-revertant102 (T-G)108Q262AThumb/alph H <i>n vitro</i> PMA- <i>lacZa</i> $\uparrow 4 X$ 144R277AThumb/alph H <i>n vitro</i> PMA- <i>lacZa</i> $\uparrow 3 X$ 144R277AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145Q278AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145L279AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145L28AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145L278AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145L28AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145K281AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145Q278AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145R284AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145R284AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145R287AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145R284AThumb/alph I <i>n vitro</i> PMA- <i>lacZa</i> No Change145R284AThumb/alph	M184A	YMDD	Misinsertion	1.6X (-T)	140
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	M184L	YMDD	Mismatch extension	↓11.1X (A-C)	136
F227APrimer gripMisinserion $1 > 5X$ (A-A)136W229APrimer gripMisinserion $1 > 5X$ (A-A)107M2301Primer gripMisinserion $1 > 5X$ (A-A)107M2301Primer gripMisinserion $1 > 1 > X$ 108W2304Primer grip/MTPMisinserion $1 > 1 > X$ 108Y115WBinding-revertant $1 > X$ 144144W266AThumb/alpha HIn vitro/FMA - lacZa $1 > X$ 144R277AThumb/alpha IIn vitro/FMA - lacZaNo Change145Q278AThumb/alpha IIn vitro/FMA - lacZaNo Change145L279AThumb/alpha IIn vitro/FMA - lacZaNo Change145Q278AThumb/alpha IIn vitro/FMA - lacZaNo Change145L280AThumb/alpha IIn vitro/FMA - lacZaNo Change145K281AThumb/alpha IIn vitro/FMA - lacZaNo Change145K281AThumb/alpha IIn vitro/FMA - lacZaNo Change145R28AThumb/alpha IIn vitro/FMA - lacZaNo Change145K281AThumb/alpha IIn vitro/FMA - lacZaNo Change145R28AThumb/alpha IIn vitro/FMA - lacZaNo Change145R28AThumb/alpha IIn vitro/FMA - lacZaNo Change145R28AThumb/alpha IIn vitro/FMA - lacZaNo Change145K287AThumb/alpha IIn vitro/FMA - lacZaNo Change145K2				↓ >26X (A-G)	
$F221A$ Primer gripMisinserion $\uparrow 5SX$ (A-A) $107$ W239APrimer gripMisinserion $\downarrow 1, SX$ $107$ M230IPrimer gripMisinserion $\downarrow 1, SX$ $108$ M230I/Primer grip/dNTPMisnerion $\downarrow 1, 3X$ (T-G) $108$ M230I/Primer grip/dNTPMisnerion $\downarrow 1, 3X$ (T-G) $108$ M230I/Primer grip/dNTPMisnerion $\downarrow 1, 3X$ (T-G) $108$ M13WBinding revertant $\downarrow 1, 3X$ (T-G) $108$ C262AThumb'alpha HIn vitroFMA -lacZa $\uparrow 3 X$ $144$ R277AThumb'alpha IIn vitroFMA -lacZaNo Change $145$ Q278AThumb'alpha IIn vitroFMA -lacZaNo Change $145$ L279AThumb'alpha IIn vitroFMA -lacZaNo Change $145$ L28AThumb'alpha IIn vitroFMA -lacZaNo Change $145$ K281AThumb'alpha IIn vitroFMA -lacZaNo Change $145$ L282AThumb'alpha IIn vitroFMA -lacZaNo Change $145$ R284AThumb'alpha IIn vitroFMA -lacZaNo Change $145$ K287AThumb'alpha IIn vitroFMA -lacZaNo Change $145$ HV-2IncertantIn vitroFMA -lacZaNo Change $145$ L74VFingersMismatch extension $45X$ (A-C) $106$ E89GFingersMismatch extension $45X$ (A-C) $106$ L74VFingersMismatch extension $48X$ $106$ E89G/S215Y <td></td> <td></td> <td>Misinsertion</td> <td>↓ &gt;9X (T-G)</td> <td>136</td>			Misinsertion	↓ >9X (T-G)	136
W29APrimer gripMisnerion $\downarrow 1.5X$ 107M2301Primer gripMisnerion $\uparrow 16X$ (Tr-G)108M230VPrimer grip(MNPMisnerion $\downarrow 1.3X$ (Tr-G)108Y115WBinding-revertantG262AThumbialpha HIn vitroFMA -lacZa $\uparrow 4 X$ 144W266AThumbialpha HIn vitroFMA -lacZa $\uparrow 3 X$ 144Q278AThumbialpha IIn vitroFMA -lacZaNoChange145Q278AThumbialpha IIn vitroFMA -lacZaNoChange145Q278AThumbialpha IIn vitroFMA -lacZaNoChange145Q28AThumbialpha IIn vitroFMA -lacZaNoChange145C280AThumbialpha IIn vitroFMA -lacZaNoChange145K281AThumbialpha IIn vitroFMA -lacZaNoChange145K281AThumbialpha IIn vitroFMA -lacZaNoChange145R284AThumbialpha IIn vitroFMA -lacZaNoChange145K287AThumbialpha IIn vitroFMA -la	F227A	Primer grip	Misinsertion	15.5X (A-A)	107
M2301Primer gripMisnerionTIGX (T-G)108M2301/Primer grip(MTP)MisnerionJ.13X (T-G)108Y115WBinding-revertant7 4 X144Q252ATumb/alpha HIn vitroFMA -lacZa $\uparrow$ 3 X144W266AThumb/alpha IIn vitroFMA -lacZaNo Change145Q278AThumb/alpha IIn vitroFMA -lacZaNo Change145L279AThumb/alpha IIn vitroFMA -lacZaNo Change145C280AThumb/alpha IIn vitroFMA -lacZaNo Change145C280AThumb/alpha IIn vitroFMA -lacZaNo Change145K281AThumb/alpha IIn vitroFMA -lacZaNo Change145K281AThumb/alpha IIn vitroFMA -lacZaNo Change145K281AThumb/alpha IIn vitroFMA -lacZaNo Change145K281AThumb/alpha IIn vitroFMA -lacZaNo Change145K287AThumb/alpha IIn vitroFMA -lacZaNo Change145K287AThumb/alpha IIn vitroFMA -lacZaNo Change145HV-2In vitroFMA -lacZaNo Change145145L74VFingersMismatch extension $\downarrow$ 6X (A-C)106E89GFingersMismatch extension $\downarrow$ 8X (A-C)106L74V/S215YFingersMismatch extension $\downarrow$ 8X (A-C)106E80G/S215YFingersMismatch extension $\downarrow$ 4.8X106L74V/S215YFingersMismatch extensi	W229A	Primer grip	Misinsertion	↓1.5X	107
M230//Primer grip/AITPMisnerionJ.3X (T-G)108W115WBinding-revertant $I$ $I$ $viro PMA - lacZa$ $\uparrow$ 4 X144W266AThumb'alpha H $In viro PMA - lacZa$ $\uparrow$ 3 X144R277AThumb'alpha I $In viro PMA - lacZa$ $\uparrow$ 3 X144Q278AThumb'alpha I $In viro PMA - lacZa$ No Change145Q278AThumb'alpha I $In viro PMA - lacZa$ No Change145Q278AThumb'alpha I $In viro PMA - lacZa$ No Change145Q280AThumb'alpha I $In viro PMA - lacZa$ No Change145C280AThumb'alpha I $In viro PMA - lacZa$ No Change145K281AThumb'alpha I $In viro PMA - lacZa$ No Change145R284AThumb'alpha I $In viro PMA - lacZa$ No Change145R284AThumb'alpha I $In viro PMA - lacZa$ No Change145K287AThumb'alpha I $In viro PMA - lacZa$ No Change145HIV-2In $In viro PMA - lacZa$ No Change145E89GFingersMismatch extension $\downarrow$ 5X (A-C)106L74VFingersMismatch extension $\downarrow$ 8.3X (A-C)106L74V/S215YFingersMismatch extension $\downarrow$ 8.3X (A-C)106E89G/S215YFingersMismatch extension $\downarrow$ 3.1X106L74V/S215YFingersMismatch extension $\downarrow$ 3.1X106E89G/S215YFingersMismatch extension	M230I	Primer grip	Misinsertion	↑16X (T-G)	108
Y115WBinding-reventatiG262ATumb/alpha HIn vitro FMA -lacZa $\uparrow$ 4 X144W266ATumb/alpha HIn vitro FMA -lacZa $\uparrow$ 3 X144R277ATumb/alpha IIn vitro FMA -lacZaNo Change145Q278ATumb/alpha IIn vitro FMA -lacZaNo Change145L279AThumb/alpha IIn vitro FMA -lacZaNo Change145C280AThumb/alpha IIn vitro FMA -lacZaNo Change145K281AThumb/alpha IIn vitro FMA -lacZaNo Change145K281AThumb/alpha IIn vitro FMA -lacZaNo Change145R284AThumb/alpha IIn vitro FMA -lacZaNo Change145R284AThumb/alpha IIn vitro FMA -lacZaNo Change145K287AThumb/alpha IIn vitro FMA -lacZaNo Change145K287AFingersM	M230I/	Primer grip/dNTP	Misinsertion	↓1.3X (T-G)	108
U202AInumbapna HIn vitroPMA - lacZa $1 4 X$ $144$ W266ATumb/alpha HIn vitroPMA - lacZa $3 X$ $144$ R277ATumb/alpha IIn vitroPMA - lacZaNoChange $145$ Q278AThumb/alpha IIn vitroPMA - lacZaNoChange $145$ Q278AThumb/alpha IIn vitroPMA - lacZaNoChange $145$ C280AThumb/alpha IIn vitroPMA - lacZa $1 5 X$ $145$ C280AThumb/alpha IIn vitroPMA - lacZa $1 5 X$ $145$ K281AThumb/alpha IIn vitroPMA - lacZaNoChange $145$ L282AThumb/alpha IIn vitroPMA - lacZaNoChange $145$ R284AThumb/alpha IIn vitroPMA - lacZaNoChange $145$ K287AThumb/alpha IIn vitroPMA - lacZaNoChange $145$ K287AThumb/alpha IIn vitroPMA - lacZaNoChange $145$ HV-2InIn vitroPMA - lacZaNoChange $145$ E89GFingersMismatch extension $\sqrt{1}(X - C)$ $106$ L74VFingersMismatch extension $\sqrt{1}(X - C)$ $106$ L74V/S215YFingersMismatch extension $\sqrt{1}(3$	r i i bw	Binding –revertant	L IN TMA 1-7	1 4 V	144
W26AIhumbalpha HIn vitroFMA - lacZa $\uparrow$ 3 X144R277AThumb'alpha IIn vitroFMA - lacZaNoChange145Q278AThumb'alpha IIn vitroFMA - lacZaNoChange145L279AThumb'alpha IIn vitroFMA - lacZaNoChange145C280AThumb'alpha IIn vitroFMA - lacZaNoChange145C280AThumb'alpha IIn vitroFMA - lacZaNoChange145K281AThumb'alpha IIn vitroFMA - lacZaNoChange145R284AThumb'alpha IIn vitroFMA - lacZaNoChange145R284AThumb'alpha IIn vitroFMA - lacZaNoChange145K287AThumb'alpha IIn vitroFMA - lacZaNoChange145K287AThumb'alpha IIn vitroFMA - lacZaNoChange145K287AThumb'alpha IIn vitroFMA - lacZaNoChange145HIV-2InIn vitroFMA - lacZaNoChange145L74VFingersMismatch extension $\downarrow$ 5X (A-C)106E89GFingersMismatch extension $\downarrow$ 6X (A-C)106L74V/S215YFingersMismatch extension $\downarrow$ 13X106E89G/S215YFingersMismatch extension $\downarrow$ 13X106MLVV223APalm/YVDDMisinsetion - RNA $\uparrow$ 79,5X (T-C)105	G262A	Thumb/alpha H	In vitroFMA –lacZa	4 X	144
$\kappa_{L'/A}$ Inumb appa 1In vitro FMA - lacZaNo Change145Q278ATumb alpha 1In vitro FMA - lacZaNo Change145L279ATumb alpha 1In vitro FMA - lacZaNo Change145C280ATumb alpha 1In vitro FMA - lacZaNo Change145K281ATumb alpha 1In vitro FMA - lacZaNo Change145L282AThumb alpha 1In vitro FMA - lacZaNo Change145R284AThumb alpha 1In vitro FMA - lacZaNo Change145G285AThumb alpha 1In vitro FMA - lacZaNo Change145K287AThumb alpha 1In vitro FMA - lacZaNo Change145HV-2In vitro FMA - lacZaNo Change145L74VFingersMismatch extension $4S$ (A-C)106E89GFingersMismatch extension $4S$ (A-C)106S215YFingersMismatch extension $4_S$ (A-C)106L74V/S215YFingersMismatch extension $4_S$ (A-C)106E89G/S215YFingersMismatch extension $4_S$ (A-C)106WLVWLWIMismatch extension $4_S$ (A-C)106WLVMLVMismatch extension $4_S$ (A-C)105	W200A	Thumb/alpha H	IN VIITO FMA – lacZa	1 3 X	144
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HV-2     Instruction activity     Instruction activity     Instruction activity     Instruction activity       L74V     Fingers     Mismatch extension     \$1,1X     106       E89G     Fingers     Mismatch extension     \$6X (A-C)     106       S215Y     Mismatch extension     \$4,3X (A-C)     106       L74V/\$215Y     Fingers     Mismatch extension     \$13X     106       E89G(S215Y     Fingers     Mismatch extension     \$13X     106       MLV     V223A     Palm/YVDD     Misinsertion - RNA     \$79,5X (T-C)     105	K287A	Thumb/alpha I	In vitro FMA $-lacZa$	NoChange	145
L74V         Fingers         Mismatch extension         ↓1.1X         106           E89G         Fingers         Mismatch extension         ↓6X (A-C)         106           S215Y         Mismatch extension         ↓8.3X (A-C)         106           L74V/S215Y         Fingers         Mismatch extension         ↓-3.3X         106           L74V/S215Y         Fingers         Mismatch extension         ↓-13X         106           L89G/S215Y         Fingers         Mismatch extension         ↓-13X         106           MLV         7223A         Palm/YVDD         Misinsertion – RNA         ↑79.5X (T-C)         105	HIV-2				
E89G     Fingers     Mismatch extension $4x$ (A-C)     106       \$215Y     Mismatch extension $4x$ (A-C)     106       174V/S215Y     Fingers     Mismatch extension $4x$ (A-C)     106       L74V/S215Y     Fingers     Mismatch extension $4x$ (A-C)     106       MLV     V223A     Palm/YVDD     Misinsertion - RNA $7p_{5X}$ (T-C)     105       Misinsertion - RNA $7p_{4x}$ (T_C)     105	L74V	Fingers	Mismatch extension	↓1.1X	106
S215Y         Mismatch extension         48.3X (A-C)         106           L74V/S215Y         Fingers         Mismatch extension         4-13X         106           L74V/S215Y         Fingers         Mismatch extension         4-13X         106           MLV         V223A         Palm/YVDD         Mismetrion – RNA         ↑79.5X (T-C)         105	E89G	Fingers	Mismatch extension	↓6X (A-C)	106
L74V/S215Y         Fingers         Mismatch extension         \$\phi13X\$         106           E89G/S215Y         Fingers         Mismatch extension         \$\phi4.8X\$         106           MLV         V223A         Palm/YVDD         Mismetion – RNA         \$\phi9.5X (T-C)\$         105	\$215Y	e	Mismatch extension	↓8.3X (A-C)	106
E89G/\$215Y         Fingers         Mismatch extension         ↓4,8X         106           MLV         V223A         Palm/YVDD         Misinsertion – RNA         ↑79,5X (T-C)         105           Misinsertion – RNA         ↑4,4X (T,C)         105	L74V/S215Y	Fingers	Mismatch extension	↓>13X	106
MLV         The second sec	E89G/S215Y	Fingers	Mismatch extension	↓4.8X	106
V223A Palm/YVDD Misinsertion – RNA <sup>(†79,5X</sup> (T-C) 105 Misinsertion – RNA <sup>(†14,14X)</sup> (T <sub>2</sub> C) 105	MLV	<i>a</i>		*	
Misinsertion – RNA 141 4X (T-C) 105	V223A	Palm/YVDD	Misinsertion - RNA	↑79.5X (T-C)	105
			Misinsertion - RNA	141.4X (T-C)	105

## **Retroviral Mutation Rates**

Mutation type	Wild Type RT <sup>a</sup> Number of Mutants/ Total	Wild Type RT mutant frequency <sup>b</sup>	Y586F RT <sup>a</sup> Number of Mutants/ Total	Y586F RT mutant frequency <sup>b</sup>	Relative increase in mutant frequency <sup>c</sup>
Substitutions near A-Tracts <sup>d</sup>	7/51	0.15%	34/60	2.59%	17.2
Other substitutions <sup>e</sup>	19/51	0.39%	8/60	0.61%	1.6
All substitutions	26/51	0.54%	42/60	3.19%	5.9
Frameshifts and template switching mutations	25/51	0.51%	18/60	1.36%	2.7
All mutations	51/51	1.05%	60/60	4.55%	4.3

Tuble 5. Effect of what type and 15001 mutant it is on 1 requencies of 5 dostitutions (data taken from (10+))
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<sup>a</sup> Number of mutants containing GFP-inactivating mutations identified by DNA sequencing containing a particular type of mutation / total number of mutants containing GFP-inactivating mutations identified by DNA sequencing. <sup>b</sup> Mutant frequencies determined by multiplying the proportion of mutants sequenced by the overall mutant frequency (e.g., the mutant frequency for substitutions near A-tracts for the wild-type RT is (7/51) x 1.05% = 0.15%). <sup>c</sup> Fold increase in mutant frequency for the Y586F mutant relative to wild-type RT (e.g., the relative increase in the frequency of substitutions near A-tracts is 2.59%  $\div$  0.15% = 17.2). <sup>d</sup> Substitution for which there was an A-tract (AAAA, TTTT, or AATT) within 18 nt of the mutation site. <sup>e</sup> Substitution for which an A-tract was not present within 18 nt of the mutation site.

R. RNR-dependent DNR synthesis
TAAAGATCCCAACGARARGAGAGACCACATGGT
TAAAGATCCCAACGARARGAGAGACCACATGGT
TGAAGCAGCATGACtTTTTCAAGAGCGCCATGC
GAAAGCTCACCCTGARATTCATCTGCACCACTG
CACTGGCGTGGTCCCARTTCTCGTGGAACTGGA
GGAAAGCTCACCCTgARATTCATCTGCACCACT
CATATGAAGCAGCAtGACTTTTTCAAGAGCGCC
CTATGTGCAGGAGAGAGAACCATCTTTTTCAAAGA
CTATGTGCAGGAGAGAGACCATCTTTTTCAAAGA
GCT AT GT GC AGG AG a GA ACC AT CT TT T C A A A G
GCT AT GT GC AGG AG ⊒G AACC AT CT TT T C AAAG
GCT AT GT GC AGG AG AGAACC AT CT TT T C AAAG
TGGATGGCGAcGaGgATGGGCACARATTTTCTG
CT GGAT GGC GAT GT gAAT GGGC AC ARATTTT CT
TACTACCTTCACCT_TGGCGTGCAGTGCTTTTC
TGATGCCACATACGgAAAGCTCACCCTGARRTT
TGATGCCACATACGgAAAGCTCACCCTGARRTT
TCACTACCTTCACCEATGGCGTGCAGTGCTTT
B. DNR-dependent DNR synthesis
CATGGCCGACAAGCAAAGAAAGGAATGGCATCAAGGT
ATGGCGTGCAGTGCTTTTCCAGATACCCAGACC
ATGGCGTGCAGTGCTTTTCCAGATACCCAGACC
CTGGCGTGGTCCCRATTC+CGTGGAACTGGATG
GTGGTCCCAATTCTCGTGGAACTGGATGGCGAT
TGCAGTGCTTTTCCAGAT=CCCAGACCATATGA
TGCAGTGCTTTTCCAGAT CCCAGACCATATGA
ACCATCTTTTTCAAAGATgACGGGAACTACAAG
ACAAGCRRRRGAATGGCAtCAAGGTCAACTTCA
ACAAGCARARGAATGGCA <del>U</del> CAAGGTCAACTTCA
CCATCTTTTTCAAAGATGaCGGGAACTACAAGA
AACGRARAGAGAGACCAC TGGTCCTGCTGGAG
GACTTTTTCAAGAGCGCCATGCCCGAGGGCTAT
CRARAGAATGGCATCAAGgTCAACTTCAAGATC
TTTTCTGTCAGCGGAGAGgGTGAAGGTGATGCC
ARTTCATCTGCACCACTGGAAAGCTCCCTGTGC

**Figure 4.** Substitution mutations in GFP induced by the Y586F mutant RT are in regions containing A-tracts. The substituted nucleotides are shown in lower case letters and shaded. The A-tract sequences (AAAA, TTTT, and AATT) that are within 18-nt of the site of substitution are shown in bold type. Assuming that the A-tracts must be in contact with the RT to have an effect on accuracy of DNA synthesis, substitutions that occurred 5' of the A-tracts occurred during RNA-dependent DNA synthesis (A) and substitutions that occurred 3' of the A-tracts occurred during DNA-dependent DNA synthesis (B). Data taken from reference (104).

conformation of the template-primer complex appears to be a significant structural determinant of fidelity. It was hypothesized that the wild-type RT evolved to facilitate a proper conformation of the template-primer that is amenable to incorporation of the correct nucleotides at the polymerase active site. When wild-type RT encounters irregular template-primer conformations such as those induced by the presence of A-tracts, certain structural determinants of RT facilitate an alteration of the templateprimer conformation that is necessary for fidelity of DNA synthesis. It was proposed that the Y586 residue and the RNase H primer grip region is a structural determinant of RT that is important for inducing a conformation of the template-primer duplex that is necessary for accuracy of DNA synthesis. When Y586 is substituted with F, it is no longer able to facilitate this template-primer conformation when A-tracts are present within 18 nt of the site of polymerization; as a result, the rate of substitutions is increased in the vicinity of A-tracts.

What is the nature of the template-primer conformation that is necessary for accurate DNA synthesis? The structures of the RNA:DNA and DNA:DNA hybrids in complex with HIV-1 RT have been determined (figure 3). These structures indicate that both hybrids possess A-form structure near the polymerase active site, a 41-degree bend, followed by B-form DNA near the RNase H active site. Aform conformation of the template-primer has been shown to be present near the active sites of other polymerases and is believed to contribute to fidelity by reducing the impact of sequence- dependent structural alterations on fidelity. In contrast, the presence of B-form DNA near polymerase active sites is associated with low fidelity and mutational hotspots (113). A-form DNA has a wider minor groove, which can provide more access for the RT to make contacts with the template-primer near the active site. Alterations in

the template-primer conformation can change the structure of the polymerase active site and have an impact on the ability of the polymerase to discriminate between correct and incorrect nucleotide substrates.

To summarize, these results have identified two important features of the reverse transcription complex that are important for accuracy of DNA synthesis. First, the conformation of the template-primer is an important determinant of fidelity; the A-form template-primer duplex that is present near the polymerase active site appears to be an important feature of the polymerase active site and perhaps is critical for accurate DNA synthesis. Second, the Y586 residue and the MLV RNase H primer grip are important structural elements that appear to be critical for maintaining a proper template-primer conformation near the polymerase active site, even when irregular template primer conformations such as A-tracts are encountered.

## 9. FUTURE DIRECTIONS

Genetic variation in HIV-1 populations has played a central role in our ability to deal with the AIDS epidemic by contributing to rapid emergence of drug resistance and escape from immune responses. A greater understanding of the mechanisms that contribute to RT fidelity could lead to novel antiviral strategies. In the future, it will be desirable to use a variety of experimental approaches to ascertain the retroviral mutation rates that will provide meaningful results that can be applied to a replicating HIV-1 virus in an infected patient. In this regard, it would be challenging but important to perform these experiments in cells that are natural targets for the viral infection. Another challenging task is to determine the contribution of RNA polymerase II-mediated RNA transcription to retroviral variation. The structural determinants of RT that are important for fidelity in vivo are now beginning to be elucidated. It will be necessary to take advantage of the available structural information on HIV-1 RT and apply it to the understanding of mechanisms of RT fidelity. Finally, the newly discovered role of the template-primer duplex structure and the RNase H primer grip domain in the fidelity of reverse transcription should be fully explored.

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**Key Words:** Retrovirus, Mutation Rate, Genetic Variation, Fidelity, Reverse Transcriptase, RNase H Primer Grip, Template-Primer Structure, dNTP-Binding Site, Misinsertions, Mismatch Extension, RNA Polymerase II, Transcription Fidelity, Review

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