High Fidelity of Internal Strand Transfer Catalyzed by Human Immunodeficiency Virus Reverse Transcriptase*

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A system to study the fidelity of internal strand transfer events was constructed. A donor RNA, on which reverse transcriptase (RT)-directed DNA synthesis was initiated, shared homology with an acceptor RNA, to which DNAs initiated on the donor could transfer. The homology occurred over a 119-base internal region of the donor which coded for the N-terminal portion of the α-lac gene. Polymerase chain reaction (PCR) was used to amplify DNA synthesis products. The PCR products were then digested with PvuII and EcoRI and ligated into a vector which had this same region excised. Transformed Escherichia coli were screened for the ability to produce a functional β-galactosidase protein by blue-white phenotype analysis with white colonies scored as those with errors in α-lac. Products synthesized on the donor were used to assess the error rate of human immunodeficiency virus-RT while products transferring to and subsequently extended on the acceptor (transfer products) were used to monitor transfer fidelity. Human immunodeficiency virus-RT made approximately 1 error per 7500 bases copied in the assay. Nucleocapsid protein (NCp), although stimulating strand transfer 3-fold, had no effect on RT fidelity. Transfer products in the absence of NCp had essentially the same amount of errors as donor-directed products while those produced with NCp showed a slight increase in error frequency. Overall, strand transfer events on this template were highly accurate. Since experiments with other templates have suggested that transfer is error prone, the fidelity of strand transfer may be highly sequence dependent.

The human immunodeficiency virus (HIV) has been shown to contain a high degree of genetic heterogeneity (1). The high error rate of HIV reverse transcriptase (RT) (2–8), and recombination between viral genomes (for reviews, see Refs. 9 and 10) are major contributing factors in the generation of diversity. Retroviruses are single-stranded plus sense RNA viruses which DNAs initiated on the donor could transfer. The homology occurred over a 119-base internal region of the donor which coded for the N-terminal portion of the α-lac gene. Polymerase chain reaction (PCR) was used to amplify DNA synthesis products. The PCR products were then digested with PvuII and EcoRI and ligated into a vector which had this same region excised. Transformed Escherichia coli were screened for the ability to produce a functional β-galactosidase protein by blue-white phenotype analysis with white colonies scored as those with errors in α-lac. Products synthesized on the donor were used to assess the error rate of human immunodeficiency virus-RT while products transferring to and subsequently extended on the acceptor (transfer products) were used to monitor transfer fidelity. Human immunodeficiency virus-RT made approximately 1 error per 7500 bases copied in the assay. Nucleocapsid protein (NCp), although stimulating strand transfer 3-fold, had no effect on RT fidelity. Transfer products in the absence of NCp had essentially the same amount of errors as donor-directed products while those produced with NCp showed a slight increase in error frequency. Overall, strand transfer events on this template were highly accurate. Since experiments with other templates have suggested that transfer is error prone, the fidelity of strand transfer may be highly sequence dependent.

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1 The abbreviations used are: HIV, human immunodeficiency virus; RT, reverse transcriptase; RNase H, ribonuclease H; NCp, nucleocapsid protein; Tag, Thermus aquaticus; PCR, polymerase chain reaction; NCp, nucleocapsid protein; MCS, multiple cloning site.

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**Fig. 1. Construction of plasmid pBSAPvuII1146.** Plasmid pBSM13+ was manipulated as described to produce pBSAPvuII1146. The resulting plasmid had bases 1149--1155 deleted. This resulted in loss of the PvuII site spanning 1146 (start of site)--1151. Numbering is based on the map for pBSM13+ (Stratagene).

β-galactosidase. Reverse transcriptase-derived DNA products were PCR-amplified and tested in an α-complementation assay. Errors occurring during RNA-directed DNA synthesis or strand transfer were scored based on the inability of mutated products to complement β-galactosidase activity, resulting in white rather than blue colonies in the assay. Results showed that strand transfer and RNA-directed DNA synthesis were of approximately equal fidelity. The presence of viral nucleocapsid protein (NCp) enhanced strand transfer but had little effect on fidelity. This suggests that transfer on this substrate is a highly accurate process. Taken together with results from other technologies, our work implies that the fidelity of strand transfer may be highly sequence dependent.

**EXPERIMENTAL PROCEDURES**

**Materials**

Recombinant HIV-RT, having properties described (31), was graciously provided to us by Genetics Institute (Cambridge, MA). This enzyme had a specific activity of approximately 40,000 units/mg (1 unit of RT is defined as the amount required to incorporate 1 nmol of dTTP in the assays). As we have previously reported, the enzyme preparations contained very low levels of single strand nuclease activity (24). Some gels were dried and used for product quantitation by phosphoimagery using a Bio-Rad GS-525 PhosphorImager.

RNA-directed DNA Synthesis with the Klenow Fragment of DNA Polymerase I—These assays were performed essentially as described above with the following changes: 1) 5 units of Klenow were used in the reactions. Reactions included the following reagents at the indicated final concentrations: 50 mM Tris-HCl (pH 8.0), 80 mM KCl, 100 mM MgCl₂, 0.1 mM EDTA (pH 8.0), 0.1 mM xylene cyanol, 0.1% bromphenol blue) containing 0.5 μg of RNase (DNase free). Samples were heated to 65 °C for 10 min to digest the RNA and then for 2 min at 90 °C. Samples were electrophoresed on 8% denaturing polyacrylamide gels as described below. Wet gels were exposed to film and transfer or full-length donor-directed product were excised and eluted (24). Some gels were dried and used for product quantitation by phosphoimagery using a Bio-Rad GS-525 PhosphorImager.

**Methods**

**Strand Transfer and DNA Synthesis Reactions with HIV-RT—**Donor template RNA (2 nt, see Fig. 2B), this was hybridized to 5'-32P-labeled primer DNA as described below. This primer-template was preincubated for 5 min at 37 °C in the presence or absence (as indicated) of 10 nM RNA acceptor template and presence or absence of NCp (2 μM final concentration), in 21 μl of buffer (see below). Four μl of HIV-RT (10 units (approximately 85 nm final concentration)) in 50 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol, and 80 mM KCl (buffer A) was added to initiate the reactions. Reactions included the following reagents at the indicated final concentrations: 50 mM Tris-HCl (pH 8.0), 80 mM KCl, 6 mM MgCl₂, 1 mM dithiothreitol, 0.1 mM EDTA (pH 8.0), 5 mM AMP, 100 μM ZnCl₂, 100 μM dNTPs, and 0.4 units/ml RNase inhibitor. Reactions were incubated for 40 min at 37 °C and stopped by addition of 25 μl of 2× formamide dye (90% formamide, 10 mM EDTA (pH 8.0), 0.1% xylene cyanol, 0.1% bromphenol blue) containing 0.5 μg of RNase (DNase free). Samples were heated to 65 °C for 10 min to digest the RNA and then for 2 min at 90 °C. Samples were electrophoresed on 8% denaturing polyacrylamide gels as described above. Gel Electrophoresis—Denaturing 8% polyacrylamide sequencing gels (19:1 acrylamide:bis-acrylamide), containing 7 M urea, native 8% polyacrylamide gels (29:1 acrylamide:bis-acrylamide), and 1% agarose gels were prepared and subjected to electrophoresis as described (33).

**Preparation of RNAs—**Run-off transcription was done as described in the Promega Protocols and Applications Guide (1989). For the donor template, plasmid pBS∆MCS, prepared as described below, was cleaved with BglII and T3 RNA polymerase was used to prepare run-off RNA transcripts approximately 189 nucleotides in length. For the acceptor template, plasmid pBSAPvuII1146 was cleaved with PvuII and T3 RNA polymerase was used to prepare run-off transcripts approximately 179 nucleotides in length. The transcription reactions were extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with ethanol. The RNA was gel-purified on denaturing polyacrylamide gels, located by ultraviolet shadowing, and recovered as described previously (24). The amount of recovered RNA was determined spectrophotometrically from optical density.

**Polymerase Chain Reaction (PCR) and DNA Sequencing—**Strand transfer products from reactions performed with acceptor template, or full-length donor-directed products from reactions performed without acceptor, were excised and eluted from denaturing gels as described above. The eluted DNA was amplified by PCR using the following primers: 5'-CCCTCTTCTTACAGTCCGCGACG-3' and 5'-GCTCGAATTCTGCACCATGCTAC-3'. The first is identical to the primer used to

**Polymerase Chain Reaction (PCR) and DNA Sequencing**
prime the donor RNA while the second overlaps the EcoRI site on the donor and acceptor (see Fig. 2B). Reactions were performed in 100 μl of 10 mM Tris-HCl (pH 9 at 25 °C), 50 mM KCl, 2 mM MgCl₂, 0.1% Triton X-100, 200 μM dNTP, 100 pm of each primer, and 5 units of Taq polymerase. Thirty-five cycles of 94 °C (1 min), 50 °C (1 min), then 72 °C (1 min) were performed. Products were extracted, precipitated, and from denaturing polyacrylamide gels (see Fig. 3) and processed as described. For details refer to "Methods."

**RESULTS**

**System Used to Study Strand Transfer—**The templates used to study strand transfer and the plasmids used to make those templates are shown in Fig. 2. The donor RNA (produced from pBSAMCS (Fig. 2A), on which RT initiates DNA synthesis, was primed with a specific 20-nucleotide DNA oligonucleotide labeled at the 5'-end with 32P. Extension to the donor would produce a 152-nucleotide product while homologous transfer of the growing DNA to the acceptor (produced from pBSΔPvuI1146) and subsequent extension yields a 199-base product (Fig. 2B). The region of homology (transfer zone) between the donor and acceptor encompassed 119 bases corresponding to a region of α-lac near the N terminus of this protein. Transfer or full-length donor-directed products were isolated from denaturing polyacrylamide gels (see Fig. 3) and used for PCR as described under "Methods." The products were processed as shown in Fig. 2B (see "Methods" for details). The final 119-base pair product, which was dephosphorylated at the EcoRI cleaved end, was ligated into plasmid pBSΔPvuI1146 (Fig. 2A) which had this same fragment excised and was dephosphorylated at the PvuII cleaved end. The plasmid and insert were dephosphorylated to decrease the likelihood of ligation products consisting of multiple inserts and/or plasmids. Such products would generally produce white colonies due to the orientation of the ligated products and not the sequence of the insert. After transformation, colony color was analyzed with white or faint blue colonies scored as those carrying plas-

**Fidelity of HIV Recombination**

![Fig. 3. Autoradiogram of a typical strand transfer experiment.](image-url)

Strand transfer reactions were performed in the absence or presence of acceptor (as indicated) and absence or presence of NCp (as indicated) as described under "Methods." The positions of the 152-base full-length donor-directed (F) or 199 base transfer products (T) are indicated (see Fig. 2B). On the far left DNA markers of the indicted size (bases) are shown.
| DNA source*  | Error frequency × 10^a ± S.D. b | Column 2, Bkgf | Transfer frequency × 10^c |
|-------------|--------------------------------|----------------|-------------------------|
| Full-length − NCp | 2.8 ± 0.32 | 2.4 (1.3) | 0.32 | 2.4 (1.3) |
| Transfer + NCp | 2.9 ± 0.45 | 2.5 (1.4) | 10.1 | 2.5 (1.4) |
| Full-length + NCp | 2.7 ± 0.34 | 2.3 (1.2) | 0.56 | 2.3 (1.2) |
| Transfer + NCp | 3.4 ± 0.5 | 3.0 (1.9) | 30.3 | 3.0 (1.9) |
| Klenow | 1.5 ± 0.56 | 1.1 (0) | | |

*Full-length refers to DNA products extended to the end of the donor template while transfer products transferred to, and were extended on the acceptor template. Products derived from synthesis with the Klenow fragment of Pol I were full-length.

a The error frequency per base is defined as the mutant colony frequency/99. Ninety-nine being the number of bases in the region being analyzed for mutations. The mutant colony frequency is the number of white or faint blue colonies/total colonies. Results were from three experiments.

b Background is defined as the error frequency obtained from insertion of a “non-mutated” insert which was homologous to the inserts derived from the different DNA sources by PCR (see “Experimental Procedures”). This frequency was 4.3 × 10^-5 or about 1 error per 23,000 bases. In parentheses are the results obtained when the value for Klenow in the second column is used as background (test).

c The transfer frequency per base was determined from the length of the transfer zone (119 bases) and the efficiency of transfer (transfer products/full-length + transfer products). The transfer efficiencies or or + NCp were 0.12 and 0.36, respectively. The reciprocal of these values × 119 is the frequency of transfer per base.

**Table I**

Compilation of results from three independent experiments

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**Frameshifts, which disrupt the reading frame of α-lac, are generally detected while base changes that result in amino acid changes may be detected depending on their effect on β-galactosidase activity. Base changes in the wobble base position that do not change amino acids will not be detected.

There are several “trivial” ways that the α-lac regions of the transformed plasmids could have been disrupted. These include ligation product multimers, T3 RNA, or Taq polymerase-derived errors, or contaminating nuclease activities that modified the insert or vector during preparation. In order for the assay to be sensitive enough to calculate HIV-RT-derived errors, it is important that the sum of all these errors be considerably less than the errors derived from HIV-RT. Background mutant colonies resulting from restriction enzyme digests and ligation procedures were estimated by cleaving the PvuII-EcoRI insert from plasmid pBSA/PvuI1146 using the protocol described in Fig. 2B, and ligating this insert back into the vector as described. Insert prepared in this manner were not subject to errors arising from T3 RNA polymerase, Taq or RT. Approximately 20,704 colonies were analyzed using this approach and 88 of these were white or faint blue (data not shown). The mutant colony frequency (mutants/total colonies) from this data was 4.3 × 10^-3. The error frequency per base was calculated by dividing this value by 99, the number of bases between the primers used for PCR in Fig. 2B. This is appropriate since the other 20 bases of the 119-base insert, although originally derived from RT in the protocol, would be specified in the insert by the PCR primer binding near the EcoRI site. The background error frequency was 4.3 × 10^-5 or 1 error per about 23,000 bases. Note that this is about 7.5-fold lower than the frequency after HIV-RT synthesis (Table I, second column). For reference, products produced from HIV-RT were also subject to T3 and T7 polymerase and T7. To estimate the contribution of these enzymes to the error frequency we performed RNA-directed DNA synthesis using the Klenow fragment of E. coli DNA polymerase I. Although this enzyme is a DNA-dependent DNA polymerase, it can also use RNA as a template (34). The fidelity of this enzyme on RNA was reported to be relatively high, with an estimated error frequency in the 10^-6 range (35). Also, using Klenow in an α-complementation assay similar to the one employed in this work, Ji and Loeb (8) found that Klenow was at least 4-fold more accurate than HIV-RT. When Klenow was used to reverse transcribe the donor RNA, an error frequency per base of about 1.5 × 10^-4 was calculated (see Table I). This frequency is about 50% that calculated with HIV-RT. Therefore, at least half of the errors observed when HIV-RT was used were derived from RT.

Nucleocapsid Protein Stimulates Strand Transfer—An autoradiogram from a typical strand transfer experiment is shown in Fig. 3. Full-length donor-directed (F) or transfer products (T) were excised from gels and processed as described under “Methods.” Donor-directed products were taken from assays without acceptor template. The presence of NCp (2 μM) in the reactions stimulated strand transfer about 3-fold. The efficiency of transfer, defined as the amount of transfer product divided by the sum of transfer plus full-length products × 100 ((T/T + F) × 100) was about 12% (average of three experiments) without and 36% (average of two experiments) with NCp. Note also that when NCp was used, several of the pause sites (sites on the template where premature termination occurs) evident in the absence of acceptor faded when acceptor was added. This suggests that the paused products are “chased” into transfer products by binding to the acceptor and subsequently being extended (24). The “chasing” was not observed without NCp although transfer clearly occurred. However, in the presence of higher amounts of acceptor template (40 nM), several of the pause sites decrease in intensity and the level of transfer products increases even in the absence of NCp (data not shown). Others have also shown that the level of strand transfer is proportional to the amount of acceptor (36). It is likely that chasing was not detected in Fig. 3 due to the low level of transfer products without NCp and because it only occurs in the presence of NCp. The fact that several different paused products appear to transfer suggests that strand transfer occurs from several locations on the template.

Transfer and Donor-directed Products Showed Approximately the Same Level of Errors in the Presence or Absence of NCp—Transfer and donor-directed products were processed as described in Fig. 2B. Analyses from three independent experiments are shown in Table I. In each experiment, 1500 to several thousand colonies derived from a given DNA source were scored. Error frequencies for transfer and donor-directed products produced using standard conditions did not vary significantly. As was previously noted, products produced with Klenow had significantly less errors. We also performed assays using suboptimal concentrations of dATP during HIV-RT synthesis. The error frequency increased about 10-fold when 1 μM dATP was used as opposed to 100 μM in the standard assay (data not shown). These results indicate that a decrease in fidelity can be detected by the assay. The only notable deviation for HIV-derived products was a small increase in the error frequency for transfer products in the presence of NCp. The third column shows the results after subtraction of background (see definition in table legend) or subtraction of the error frequency obtained using Klenow (in parentheses). These values reflect the highest and lowest error frequencies, respectively, for HIV-RT in the assay. The first value assumes an error frequency of zero for RNA polymerase and T7 while the second assumes a zero value for Klenow. Based on these values the detectable error frequency of RT in this assay was between 1.3 × 10^-4 and 2.4 × 10^-4 or 1 error per approximately 4200–7700 bases. This value is close to that obtained for RNA-directed DNA synthesis by Ji and Loeb (8), but is significantly higher than values from others (6, 29). Also shown in Table I are calculations for the transfer frequency. These numbers
express the frequency of transfer per base over the 119-base region of homology (transfer zone) between the donor and acceptor. The inverse of this value reflects the average number of bases within the transfer zone synthesized per strand transfer event. Without NCp there was about one transfer event per 990 bases and one per 330 bases with NCp.

**Sequence Analysis of Mutants**—Several of the mutants from donor-directed products produced in the absence of NCp or Klenow products were sequenced (Fig. 4). It is difficult to assign specific errors to RT since the background in the assay was relatively high (see above). Errors may have resulted from RNA polymerase, Taq, or improper insertion. The latter group consisted mostly of plasmids with 2 inserts in which the PvuII cut blunt end of one insert had ligated to a modified EcoRI end of a second insert. The 4 base 5’ overhang generated by EcoRI was apparently cleaved off by a contaminating nuclease generating a blunt end. This accounted for about one-fourth of the mutated plasmids observed with Klenow (data not shown). The other mutant plasmids from Klenow consisted of single base substitutions and deletions, presumably resulting from errors made by RNA polymerase, or Taq, or Klenow. Some of the observed errors were unique to assays with HIV-RT. Among them were frameshifts within a run of A’s (bases 65–68) and multiple substitution mutants at positions 61 and 86.

**DISCUSSION**

We have shown that internal strand transfer occurring over a defined region of RNA is highly accurate. This conclusion was based on the error frequency of HIV-RT DNA synthesis products produced by primer extension on a single template, or those which transferred to and were extended on a second acceptor template. Each type of product showed similar levels of errors (Table I). If strand transfer were inaccurate, then transfer products should have more errors than those that had not undergone transfer. Due to the assay background and variability, very small increases in error frequency could not be reliably detected. However, if 2% or more of transfer events were inaccurate this could have been easily detected. A 2% per event error rate for transfer would have increased the error frequency by about $2 \times 10^{-4}$ per base. This calculation is based on 2 additional mutant colonies per 100 total colonies or 2% additional mutants. Since each insert corresponds to 99 synthesized nucleotides (see “Results”) the error frequency would be $(2/100 \times 99)$ or about $2 \times 10^{-4}$. This level of error would increase the $2.8 \times 10^{-4}$ value for full-length products without NCp from Table I (column 2) to $4.8 \times 10^{-4}$ for the transfer products. Thus, if errors occurred in only 1 in 50 transfer events they would have been easily detected. There was some increase in error frequency for transfer products versus donor-directed products produced in the presence of NCp. An increase from $2.7 \times 10^{-4}$ to $3.4 \times 10^{-4}$ represents one additional error per about every 14,000 bases copied or a 0.7% per event error rate for transfer. Since this increase is relatively small it may have resulted from normal experimental variation. Several additional experiments would have to be performed to substantiate this difference. What was clear from the results is that from a per base perspective, strand transfer on this particular template was considerably more accurate than RT-directed DNA synthesis. The latter had a maximum error frequency after background subtraction of $2.4 \times 10^{-4}$ in the assay (see “Results”). This corresponds to one error per about 4200 nucleotides. Clearly, errors resulting from strand transfer were below this value. That does not mean that strand transfer is 100% accurate, but that base misincorporation errors resulting from HIV-RT’s infidelity occur at a significantly higher frequency than those resulting from erroneous strand transfer. This may be especially true in vivo where the strand transfer frequency using an spleen necrosis virus based system was estimated to be about 4% per kilobase during a single round of replication (9). If the frequency of an inaccurate transfer event was 1%, using this value of 4% to estimate the frequency of recombinant per kilobase leads to an error frequency per base of $4 \times 10^{-7}$ (0.01 x 0.04/1000) or 1 error per $2.5 \times 10^{6}$ bases on average. Our experiments suggest an error frequency per strand transfer event of less than 1%. If this scenario is reasonable, the contribution of strand transfer to base misincorporations would be essentially negligible.

We should note that the frequency of strand transfer events in our in vitro system is 1–2 orders of magnitude greater than the 4% per kilobase value cited above. We observed frequencies corresponding to average rates of about 1 transfer event per 1000 or 330 nucleotides for reactions in the absence or presence of NCp, respectively (Table I). The high rate observed in our experiments may result in part, from the small sizes of the donor and acceptor templates and the high ratio of acceptor to donor (5:1) (36). The relatively large size of the retroviral genome would likely make alignment of homologous regions more difficult than with the small templates used here. In addition, one might expect that the large number of bases of the genome may increase the likelihood of erroneous transfer events, essentially by increasing the potential targets for transferring DNAs. For example, a nascent DNA could potentially transfer to a region on the acceptor genome hundreds or even thousands of bases away from the region homologous to that used for synthesis of the DNA. Such events are referred to as “nonhomologous” strand transfer since the DNA transfers to a region of a second template different from the region on which it was synthesized. In this type of transfer there is often, but not always, a small region of complementarity between the acceptor RNA and 3’ terminus of the transferring DNA (37, 38). Experiments performed in vivo using a system based on spleen necrosis virus and Moloney murine leukemia virus indicate that nonhomologous recombination occurs at only 1/100th to 1/10000th the frequency of homologous recombination (37). Nonhomologous transfer events may be important for viral transduction (38, 39); however, homologous transfer events, being more frequent and less likely to produce defective provirus, presumably contribute more to the genetic diversity of the viral population.
The size of the templates used in our experiments limits the potential for evaluating nonhomologous transfer. In fact, any transfers resulting in the deletion or insertion of about 10 or more bases would have been missed by our assays as a result of the way the insert was isolated (by gel purification in which a region of about ±10 bases was excised). Therefore, the assay we used assesses mutations occurring during homologous strand transfer. Our conclusions suggest that such transfers are highly accurate. Others, however, have found strand transfer with HIV-RT to be somewhat inaccurate (26, 29). Using an in vitro system designed to test the fidelity of internal transfer within a hypervariable region of the nef gene, Wu et al. (29) reported that strand transfer was more error-prone than RNA-directed DNA synthesis. The overall error frequencies per base for DNA synthesis and strand transfer were $2.8 \times 10^{-5}$ and $6.2 \times 10^{-5}$, respectively. The value for RNA-directed DNA synthesis is about 10-fold lower than the value calculated from our experiments but is comparable to the values suggested by Boyer et al. (6). Although the error frequency in strand transfer more than doubled in the above experiments, the overall gross increase was $2.2 \times 10^{-5}$ or $3.4 \times 10^{-5}$. We note that such an increase would not be reliably detectable in our assays and would represent a tenuous increase over the error frequency which we determined. There were important differences between our experiments and those of Wu et al. (29). Although both used color changes due to loss of α-complementation to detect errors, in the experiments of Wu et al. (29) synthesis and transfer occurred over a hypervariable region of the nef gene which was inserted within the multiple cloning site of plasmid pBSM13+, between the α-lac promoter and the majority of the coding region for α-lac. The insert was in-frame with the downstream α-lac gene. Mutations occurring during RNA synthesis or strand transfer within the insert region would disrupt α-lac only if the mutations resulted in a termination codon or frameshift. Therefore, the assay was designed to detect mutations resulting in frameshifts only. Since our assay detected both frameshifts and some point mutations (see “Results”), we would likely detect a higher proportion of the errors. However, since a high proportion of the errors is informative with respect to analyzing specific errors made by HIV-RT, implying that they resulted from this enzyme (Fig. 4). All things considered, the lower background of the assays used by Boyer et al. (6) and Ji and Loeb (8) make these approaches more informative with respect to analyzing specific errors made by HIV-RT.

In conclusion, our work indicated that strand transfer on the template used in these experiments was highly accurate, while experiments using a different template (29) showed that some transfer events are error prone. Taken together the results imply that the fidelity of strand transfer may be highly sequence dependent.

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Fidelity of HIV Recombination

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