Supplementary Data

Tables

Table S1: The synthetic oligonucleotides used in this study.

OLIGONUCLEOTIDE DESIGNATION	SEQUENCE
I1 (21mer)	5’-GTGTGGAAAATCTCTAGCAGT -3’
I2 (21mer)	5’-ACTGCTAGAGATTTTCCACAC-3’
I3 (19mer)	5’-TGCTAGAGATTTTCCACAC-3’
I4 (18mer)	5’-GCTAGAGATTTTCCACAC-3’
I5 (17mer)	5’-CTAGAGATTTTCCACAC-3’
I6 (16mer)	5’-TAGAGATTTTCCACAC-3’
I7 (16mer)	5’-TGCTAGAGATTTTCCA-3’
I8 (40mer)	5'-GCCGGCCCATGGTCTTCCTAGAAAATATCCCCTCAGCCAC-3'
I9 (21mer)	5’-GAGTGAATTAGCCCTTCCAGT-3’
I10 (21mer)	5’-TGGAAGGGCTAATTCACTCAC-3
I11 (20mer)	5’-GTGTGGAAAATCTCTAGCAG-3’
I12 (23mer)	5’-GTGTGGAAAATCTCTAGCAGTGG-3’
I13 (19mer) (RNA)	5’-UGCUAGAGAUUUUCCACAC-3’
I14 (21mer)	5’-TGGTGGAAAATCTCTAGCATG-3’
I15 (19mer)	5’-TGCTAGAGATTTTCCACCA-3’
I16 (21mer)	5’-GCGTGGAAAATCTCTAGCAGC-3’
I17 (19mer)	5’-TGCTAGAGATTTTCCACGC-3’
I18 (21mer)	5’-GAGTGGAAAATCTCTAGCAGA-3’
I19 (19mer)	5’-TGCTAGAGATTTTCCACTC-3’
I20 (21mer)	5’-GGGTGGAAAATCTCTAGCAGG-3’
I21 (19mer)	5’-TGCTAGAGATTTTCCACCC-3’
I22(21mer)	5’-CCGTGGAAAATCTCTAGCACC-3’
I23 (19mer)	5’-TGCTAGAGATTTTCCACGG-3’
I24 (21mer)	5’-CAGTGGAAAATCTCTAGCACA-3’
I25 (19mer)	5’-TGCTAGAGATTTTCCACTG-3’
I26 (21mer)	5’-CTGTGGAAAATCTCTAGCACT-3’
I27 (19mer)	5’-TGCTAGAGATTTTCCACAG-3’
I28 (21mer)	5’-TTGTGGAAAATCTCTAGCATT-3’
I29 (19mer)	5’-TGCTAGAGATTTTCCACAA-3’
I30 (21mer)	5’-ATGTGGAAAATCTCTAGCAAT-3’
I31 (19mer)	5’-TGCTAGAGATTTTCCACAT-3’
I32 (21mer)	5’-AAGTGGAAAATCTCTAGCAAA-3’
I33 (19mer)	5’-TGCTAGAGATTTTCCACTT-3’
I34 (21mer)	5’-TGAAAGACCCCC GCTGACGGG-3’
I35 (54mer)	5’-AATGAAAGACCCCACCTGTAGGTTGGATCCTTACCCGTCAG--CGGG GGTCTTTCA-3’
I36 (21mer)	5’-biotin-GTGTGGAAAATCTCTAGCAGT-3’
I37 (23mer)	5’-biotin-GTGTGGAAAATCTCTAGCAGTGG-3’
I38 (25mer)	5’-digoxigenin-TCCTAGAAAATATCCCCTCAGCCAC-3’
I39 (22mer)	5’-GTGTGGAAAATCTCTAGCAGTG-3’

Table S2: Template primers sets used in this study.

T/P SET	LABELLED PRIMER	TEMPLATES	SCHEME
#1	I1	I3	5'*——CAGT 3' 3' CA—— GT▲ca—— gt 5'
#2	I1	I3, I8	5' *——CAGT 3' 3' CA——GT▲ca—— cg 5'
#3	I1	I8	5' *——CAGT 3' 3'▲ca—— cg 5'
#4	I8	I1, I3	5' ——CAGT 3' 3'CA——GT▲ca—— cg*5'
#5	I1	I4	5' *——GCAGT 3' 3' CA —— CG▲ca——cg 5'
#6	I1	I4, I8	5' * —— CAGT 3' 3' CA—— G▲ca—— cg 5'
#7	I1	I5	5' *—— AGCAGT 3' 3' CA —— TC ▲ca —— tc 5'
#8	I1	I6	5' *——TAGCAGT 3' 3' CA —— AT ▲ca—— at 5'
#9	I1	I5, I8	5' *——AGCAGT 3' 3' CA—— TC ▲ca—— cg 5'
#10	I11	I3	5'*——CAG 3' 3' CA —— GT▲ca—— gt 5'
#11	I1	I4, I8	5' —— CAGT 3' 3' CA—— G▲ca—— cg *5'
#12	I1	I5, I8	5' ——AGCAGT 3' 3' CA——TC ▲ca—— cg *5'
#13	I1	No template	5'*—— CAGT 3'
#14	I1	I2	5'*——CAGT 3' 3' CA —— GTCA 5'

#15	I9	I10	5’ *———— CAGT 3’ 3’ CA———— GTca ——— gt 5’
#16	I14	I15	5’ *———— CATG 3’ 3’ AC ——— GT ac——— gt 5’
#17	I20	I21	5’ *———— CAGG 3’ 3’ CC ——— GT cc——— gt 5’
#18	I16	I17	5’ * CAGC 3’ 3’ CG GT cg gt 5’
#19	I18	I19	5’ *———— CAGA 3’ 3’ CT ——— GT ct——— gt 5’
#20	I22	I23	5’ *———— CACC 3’ 3’ GG——— GT gg——— gt 5’
#21	I24	I25	5’ *———— CACA 3’ 3’ GT——— GTgt ———gt 5’
#22	I26	I27	5’ *———— CACT3’ 3’ GA ——— GTga——— gt 5’
#23	I28	I29	5’ *———— CATT 3’ 3’ AA——— GT aa——— gt 5’
#24	I30	I31	5’ *———— CAAT 3’ 3’ TA——— GT ta———gt 5’
#25	I32	I33	5’ *———— CAAA 3’ 3’ TT——— G T t t———gt 5’
#26	I39	I3	5’*———— CAGTG 3’ 3’ CAC——— GTcac——— gt 5’
#27	I1	I13	5’ *———— CAGT 3’ 3’ CA———— GUca———— gu 5’
#28	I1	I13, I8	5’ *———— CAGT 3’ 3’ CA ———— GUca———— cg 5’
#29	I36	I3, I38	5’ biotin———— CAGT 3’ 3’ CA——— GTca———— ct dig 5’
#30	I36	I38	5’ biotin———— CAGT 3’ 3’ ca———— ct dig 5’

#31	I37	I3, I38	5’ biotin————CAGTGG 3’ 3’ CA————GT▲cacc————ct dig 5’
#32	I37	I38	5’ biotin————CAGTGG 3’ 3’▲cacc————ct dig 5’
#33	I12	I3, I8	5’ *————CAGTGG 3’ 3’ CA————GT▲cacc————cg 5’
#34	I12	I8	5’ *———— CAGTGG 3’ 3’ ▲cacc————cg 5’
#35	I34	I35	5’ *TG————GG 3’ 3’ AC————————AA 5’

The designations of the labeled primers are shown for each set, along with the compatible templates and the relative positioning of the oligonucleotides in each substrate set. The ends of the primers and the first templates are indicated by capital letters and the ends of the functional templates (second templates) are indicated in lower case letters. Nicks or gaps between two adjacent templates (bottom strands) are indicated by arrow heads and an asterisks indicate the [^{32}P]5’-ends of the functional primers.

Legends to Figures

Fig. S1: The DDDP primer-extension activity of the RTs and DNA-polymerases used in the study. All reactions were performed in a final volume of 12.5µl and are identical to those described for the clamp/polymerase assay reaction (see "Materials and Methods"), with the exception that in this experiment the T/P used (set #35, see Table S2) is suitable for assaying DDDP rather than the clamp activity. MLV RT was assayed with 5mM $MgCl_2$ or 0.8mM $MnCl_2$ while Tf1 RT with 5mM $MgCl_2$ or 0.5 mM $MnCl_2$. All other indicated enzymes were assayed with 5mM $MgCl_2$.

Fig. S2: A schematic description of the ELISA-based DNA-binding assay.
The lengths of the different template primers used are not drawn to scale. The reactions were performed as described in detail in "Materials and Methods". In each T/P schematic description, the designations of the oligonucleotides are as they appear in Table S1, along with their lengths in nts (in parentheses).

Fig. S3: Template/primer requirements for an efficient clamp activity. HIV-1 RT was incubated with the indicated T/Ps and dNTPs for assaying the clamp/polymerase activity, followed by urea-PAGE analysis (see "Materials and Methods"). T/Ps designations and their schemes are indicated, see Fig. 1 for details. For each substrate, lane 1, T/P only; lane 2, T/P+RT; lane 3, T/P+RT+dNTPs.

Fig. S4: A proposed three-dimensional model for the ability of HIV-1 RT to clamp nucleic acids. Template primers with 3'-overhangs of only two nucleotides may allow HIV-1 RT to mediate and sustain the binding of a second strand (Template 2). This is followed by DNA synthesis that extends the primer. The 3D structure of HIV-1 RT used in this model (in light yellow), was found in PDB entry 1R0A (Peletskaya et al., *J. Virol* 78: 3387-3397, 2004). Van der Waals surface was added to HIV-1 RT and the original DNA template was "nicked" (by deleting the phosphate atom) between base -2 and -3 relative to the primer 3'-end. Some nucleotides were deleted for simplicity leaving 4 bases of the primer, 3 bases of template 1 and 3 bases of template 2. Residues of HIV-1 RT within 10 Å of the deleted phosphate are displayed in black. Template 1 and template 2 are presented in green and blue, respectively. The DNA primer is presented in red. This model was created using the Accelrys DS Visualizer 1.6 software.

Fig. S1

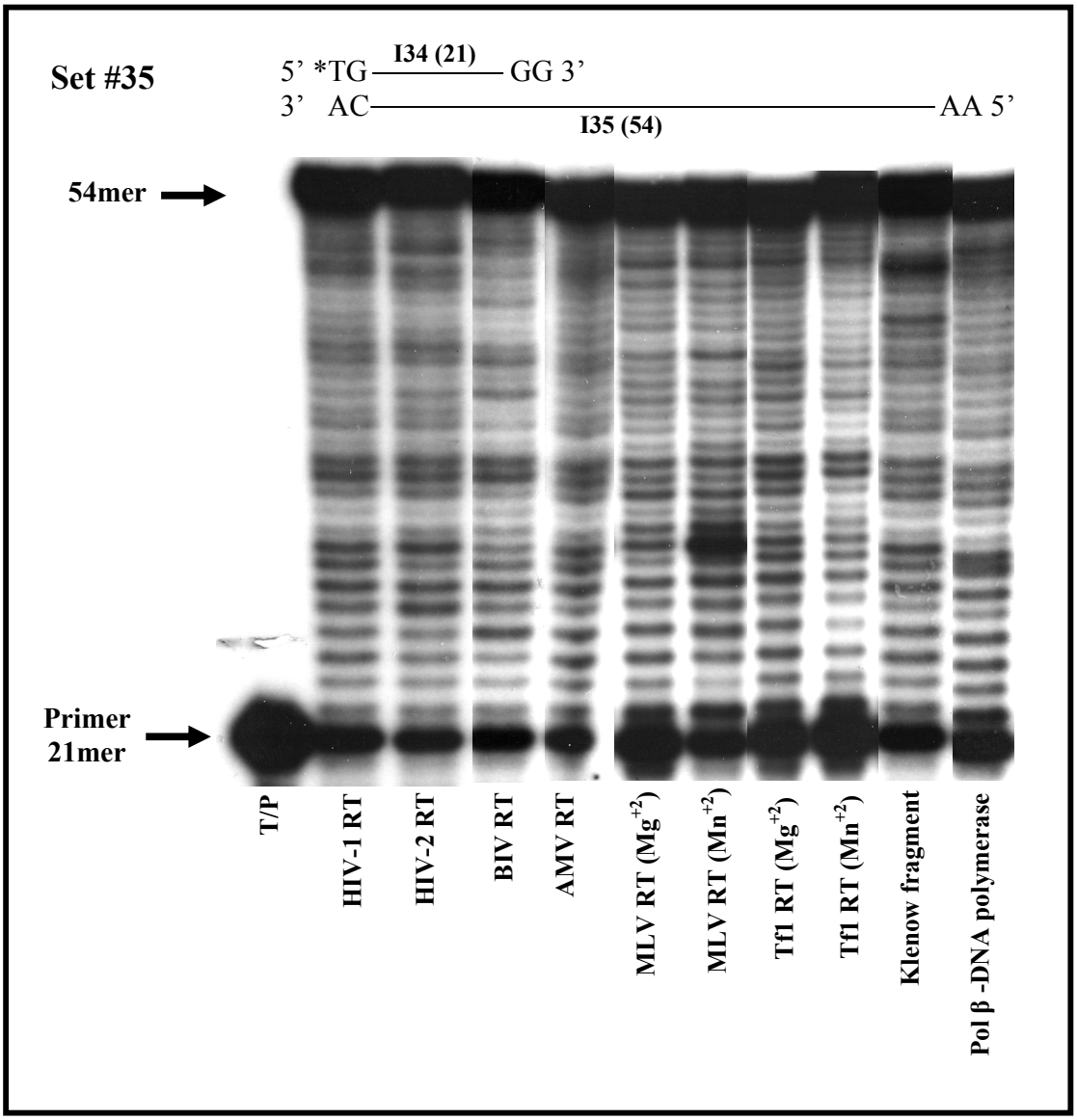

Set #35
5' *TG
I34 (21)
GG 3'
3' AC
AA 5'
I35 (54)
54mer
Primer
21mer
T/P
HIV-1 RT
HIV-2 RT
BIV RT
AMV RT
MLV RT (Mg+2)
MLV RT (Mn+2)
Tf1 RT (Mg+2)
Tf1 RT (Mn+2)
Klenow fragment
Pol β -DNA polymerase

Fig. S2

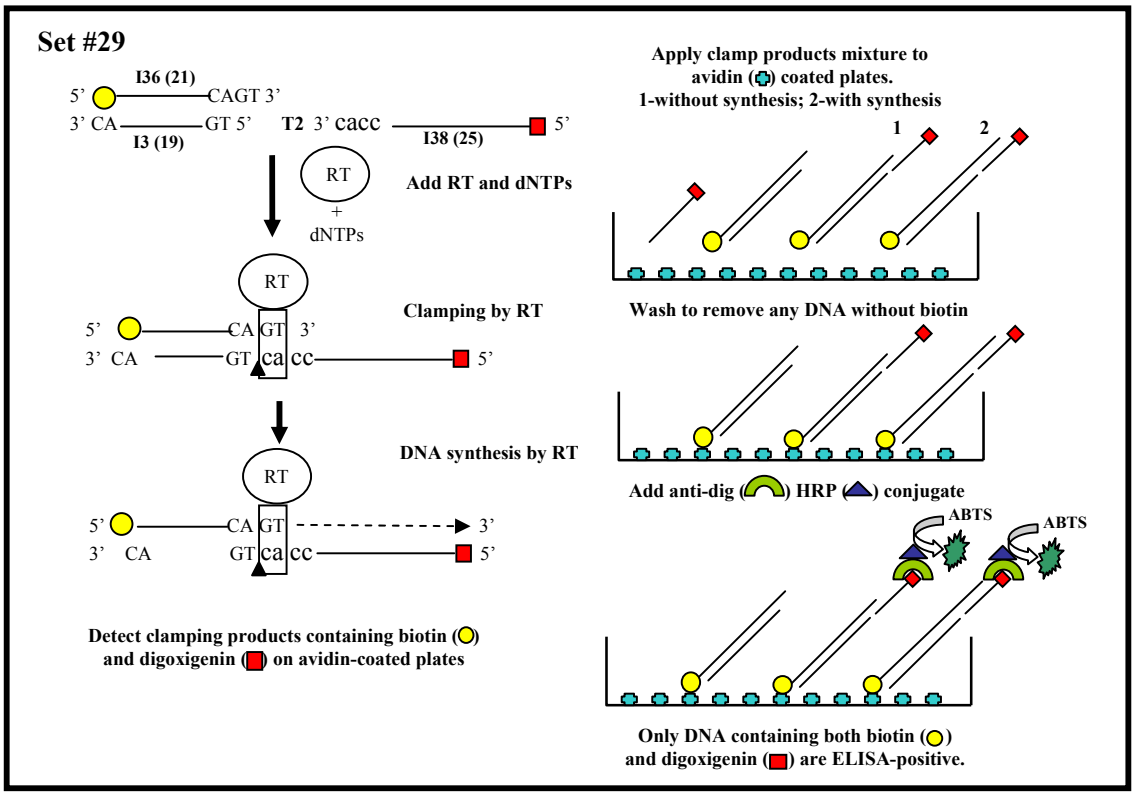

Set #29
I36 (21)
5'
CAGT 3'
3' CA
GT 5'
I3 (19)
T2 3' cacc
I38 (25)
5'
RT
+
dNTPs
Add RT and dNTPs
RT
Clamping by RT
5'
CA GT 3'
3' CA
GT ca cc
5'
RT
DNA synthesis by RT
5'
CA GT
3'
3' CA
GT ca cc
5'
Detect clamping products containing biotin ( )
and digoxigenin ( ) on avidin-coated plates
Apply clamp products mixture to
avidin ( ) coated plates.
1-without synthesis; 2-with synthesis
1
2
Wash to remove any DNA without biotin
Add anti-dig ( ) HRP ( ) conjugate
ABTS
ABTS
Only DNA containing both biotin ( )
and digoxigenin ( ) are ELISA-positive.

Fig. S3

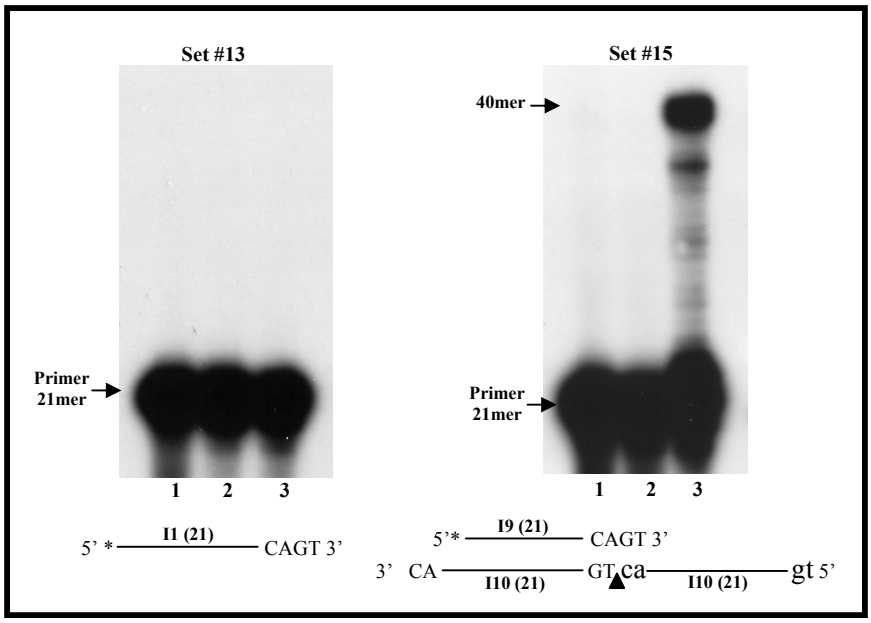
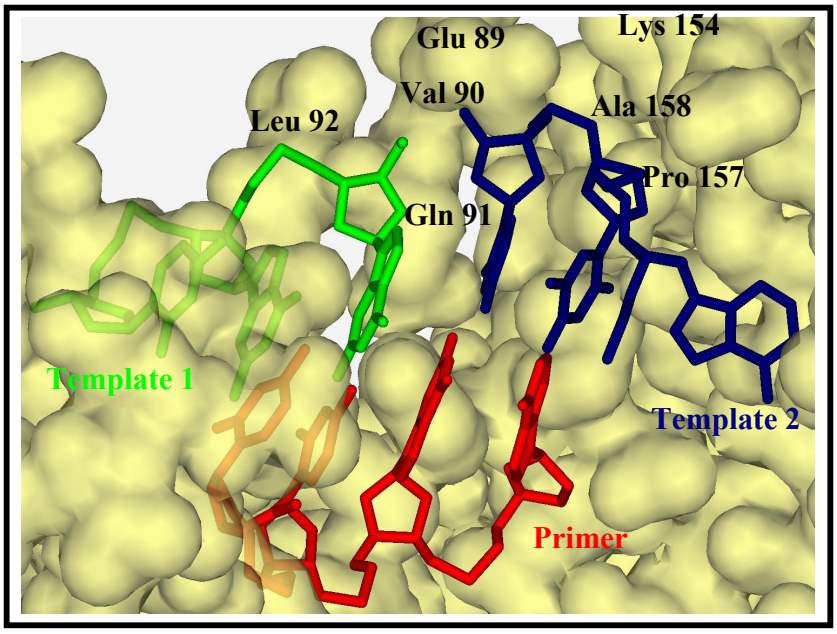

Set #13
Primer
21mer
1 2 3
5' * I1 (21) CAGT 3'
Set #15
40mer
Primer
21mer
1 2 3
5'* I9 (21) CAGT 3'
3' CA I10 (21) GTca I10 (21) gt 5'

Fig. S4


Glu 89
Lys 154
Val 90
Ala 158
Leu 92
Pro 157
Gln 91
Template 1
Template 2
Primer