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Supporting Information

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Structure and Function of an RNA-Reading Thermostable DNA Polymerase**

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Supporting Information

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Methods

Library Generation.[17] Parental DNA (pGDR11[18] *KlenTaq* (KTq) genes encoding wt, M1 and M747K) was amplified in a nested PCR (100 µl) using 0,03 u/µl Phusion polymerase (Thermo Scientific), DNA Shuffling primer forward 5'-d(CGA GGC CCT TTC GTC TTC AC)-3' and reverse 5'-d(CTT AGC TCC TGA AAA TCT CGC C)-3' (200 nM each), 200 µM of each dNTP, 3 % (v/v) DMSO and 12 fmol of the respective KTq gene. After initial denaturation for 60 s at 98 °C, 25 PCR cycles were performed with 10 s at 98 °C, 30 s at 70 °C, 60 s at 72 °C and one final elongation for 10 min at 72 °C. As a high amount of template was needed for the DNase digestion step, PCR products were gel extracted and reused as template in PCR under the same conditions (43.5 fmol template in a 100 µl reaction volume).

3 µg amplified DNA (1 µg of each amplified KTq gene) were digested with 0.33 u DNase I (Fermentas) in 10 mM Tris HCl pH 7.5, 0.1 mM $CaCl_2$ in the presence of 2.5 mM $MgCl_2$ for 1 min at 15 °C (80 µl total volume). The reaction was terminated by addition of EDTA and incubation at 65 °C for 10 min. DNA fragments in the size range of 50-200 bp were obtained.

Without further purification, 5 µL of DNA fragments were utilized in an Assembly PCR (50 µL total volume) using no primers, 200 µM of each dNTP, 3 % (v/v) DMSO and 1 u Phusion Polymerase. The fragments were reassembled using 60 cycles and an annealing temperature of 45 °C to promote recombination. After initial denaturation for 60 s at 98 °C, 60 PCR cycles were performed with 10 s at 98 °C, 30 s at 45 °C, 60 s at 72 °C and one final elongation for 10 min at 72 °C. Optimization of this step revealed less point mutations when using the Phusion polymerase instead of Taq polymerase.

A final PCR was performed with a 1:2 dilution of the Assembly PCR. 4 µL of this dilution was used as template in PCR (200 µl) using primers with restriction sites for cleavage with SphI 5'-d(CAT ACG GAT CCG CAT GCA GCC CTG GAG GAG GCC C)-3' and HindIII 5'-d(GCT CAG CTA ATT AAG CTT TCT CCT TGG CGG AGA GCC)-3' (200 nM each), 200 µM of each dNTP, 3 % (v/v) DMSO and 0.03 u/µL Phusion polymerase. After initial denaturation for 60 s at 98 °C, 20 PCR cycles were performed with 10 s at 98 °C, 30 s at 72 °C, 60 s at 72 °C and one final elongation for 10 min at 72 °C. The amplified products containing recombined full length KTq genes were gel extracted, digested with the respective restriction enzymes SphI and HindIII (Fermentas) and cloned into pGDR11 vector. The ligation reaction was transformed into *E. coli* BL21 (DE3) cells and colonies were picked randomly to generate a library containing 1,570 shuffled KTq variants. Theoretically, recombination of M1 and M747K via DNA-Shuffling comprises seven positions being either mutated or displaying the wild-type amino acid sequence, thus resulting in 2^7 = 128 mutation combination possibilities in total. Therefore, a library size of approximately 1,200 clones is required to have a 99 %

probability to sample all possible variants, presuming that every mutation combination is equiprobable.[19]

Screening. KTq variants of the generated library were expressed in 96-well plates. Cells were grown in 1 mL LB-media (per well) and protein expression was induced by addition of IPTG (1 mM final concentration) at an OD of 0.6 - 0.8. Protein expression was conducted at 37 °C for 4.5 h in LB media (carbenicillin 100 mg/L) and cells harvested afterwards. Pellets were lysed using lysozyme at a concentration of 0.1 mg/mL and digested with DNase I (0.01 mg/mL) at 37 °C for 15 min in 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 0.1 % Tween 20, 2.5 mM $MgCl_2$. After heat-denaturation at 75 °C for 45 min and centrifugation to remove bacterial cell debris, the lysates were directly used in an activity screening performed in 384-well plates using real-time PCR. Reaction mixtures (20 µL) contained 60 pM template (5'-d(CCG TCA GCT GTG CCG TCG CGC AGC ACG CGC CGC CGT GGA CAG AGG ACT GCA GAA AAT CAA CCT ATC CTC CTT CAG GAC CAA CGT ACA GAG)-3'), 250 µM of each dNTP, 750 nM of each primer (5'-d(CGT TGG TCC TGA AGG AGG AT)-3'; 5'-d(CGC GCA GCA CGC GCC GCC GT)-3'), 0.6x SYBRGreen I and 10 µL of the respective lysate in 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 0.1 % Tween 20, 2.5 mM $MgCl_2$. Initial denaturation at 95 °C for 1 min was conducted, 30 PCR cycles were performed with 10 s at 95 °C, 20 s at 55 °C and 30 s at 72 °C. Variants were further screened for reverse transcriptase activity in real-time RT-PCR, carried out in 96-well plates using MS2 RNA based on a method published earlier by Sauter *et al.*[6] DNA formation was visualized by binding of SYBRGreen I. In short, reaction mixtures (20 µL) contained 50 pg/µL MS2 RNA (Roche) as template, 200 µM of each dNTP, 100 nM of each primer (5'-d(ATC GCT CGA GAA CGC AAG TT)-3'; 5'-d(CG GAC TTC ATG CTG TCG GTG)-3'), 0.6x SYBRGreen I and 5 µL of the respective lysate in 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 0.1 % Tween 20, 2.5 mM $MgCl_2$. First, reverse transcription was conducted using an initial denaturation step of 30 s at 95 °C, an annealing step at 55 °C for 35 s and elongation for 15 min at 72 °C. Additionally, 50 PCR cycles were performed with 30 s at 95 °C, 35 s at 55 °C and 40 s at 72 °C.

KTq mutants with a ct-value lower than the ct value of the parental mutants M1 and M747K were selected and further screened for reverse transcriptase activity under more challenging conditions, including a reduction of the reverse transcription time (down to 7.5 min) in a first screening and a reduction of RNA concentration (down to 5 pg/µL) in a second screening. Variants with the most promising results in all experiments were selected and their genes sequenced at GATC-Biotech.

Proteins and Oligonucleotides. Proteins for the characterization experiments[20] and crystallization trials were purified[21] as described, respectively. Protein expression was conducted in *E. coli* BL21 (DE3) cells.

Oligonucleotides were purchased from Metabion or Biomers (Germany).

Primer Extension Assay. Reaction mixtures (20 µL) contained 150 nM radioactively labeled primer (5'-d(CGT TGG TCC TGA AGG AGG AT)-3'), 225 nM RNA template (5'-AAA UCA ACC UAU CCU CCU UCA GGA CCA ACG-3') or the respective DNA template (5'-d(AAA TCA ACC TAT CCT CCT TCA GGA CCA ACG TAC)-3'), 200 µM of each dNTP and 25 nM of the respective KTq DNA polymerase in 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 0.1 % Tween 20 and 2.5 mM $MgCl_2$. Reaction mixtures were incubated at 72 °C and terminated after 1 min by addition of 45 µl stop solution (80 % [v/v] formamide, 20 mM EDTA, 0.25 % [w/v] bromophenol blue, 0.25 % [w/v] xylenecyanol). After denaturation at 95 °C for 5 min, reaction mixtures were separated using a 12 % denaturing PAGE gel. Visualization was performed by phosphoimaging.

DNA Polymerase Activity Determination.[9] Primer extension reactions were performed at 72 °C (described as above) with an incubation time of 10 min for DNA as template and 30 min for RNA as template. Reaction mixtures (20 µl) contained 150 nM radioactively labeled primer (5'-d(CGT TGG TCC TGA AGG AGG ATA GG)-3'), 225 nM RNA template (5'-AAA UCA ACC UAU CCU CCU UCA GGA CCA ACG-3') or the respective DNA template (5'-d(AAA TCA ACC TAT CCT CCT TCA GGA CCA ACG TAC)-3'), 200 µM of each dNTP and various amounts of the respective KTq DNA polymerase in 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 0.1 % Tween 20 and 2.5 mM $MgCl_2$. Polymerase amounts present in reactions with DNA or RNA as template were 2, 1, 0.75, 0.5, 0.25, 0.125 fmol or 400, 300, 200, 150, 100, 50, 30, 20, 15, 10, 5, 3, 2, 1, 0.5, 0.3 fmol, respectively. The observed intensities of each band yielded the conversion of dNTPs in each reaction. dNTP conversion per min was then plotted against the amount of enzyme. The linear range was analyzed and slopes were obtained using linear regression yielding the specific activity of the respective enzyme.

CD-spectra Measurement and Thermal Denaturation.[21] KTq storage buffer was exchanged via dialysis over night at 4 °C with buffer containing 137 mM NaCl, 2.7 mM KCl, 10.2 mM Na_2HPO_4, 1.8 mM KH_2PO_4 (pH 7.4) using Slide-A-Lyzer Dialysis Cassettes (Thermo Scientific). CD spectra measurements were performed at 20 °C using quartz cuvettes (light path 1 mm) and 250 µL protein sample (2.2 µM). CD spectra were measured from 200 to 250 nm (50 nm/min) with 0.1 nm data intervals and were averaged from 6 scans (CD spectrometer J815). Thermal denaturation was conducted based on CD spectroscopy by following the ellipticity at the two local minima 209 and 220 nm. Data collection was carried out at every 0.1 °C with a temperature slope of 0.2 min^{-1}. The reaction was irreversible as precipitation was observed under the experimental conditions.

Crystallization and Structure Determination. Crystallization solutions containing RT-KTq 2 (6.6 mg/mL) in 20 mM Tris HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, RNA template (5'-d(AAA GGG CGC CGU GGU C)-3') (217 µM), DNA primer 5'-d(GAC CAC GGC GC)-3') (217 µM), 1 mM ddCTP, 19 mM $MgCl_2$ were incubated at 30 °C for 60 min and subsequently mixed in 1:1 ratio with the reservoir solution (100 mM Tris HCl, pH 8.5, 0.2 M magnesium formate, and 20 % PEG 8000).

Crystallization solutions with RT-KTq 2 in complex with an all DNA duplex contained 6.4 mg/mL protein in 20 mM Tris HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, DNA template (5'-d(AAA GGG CGC CGT GGT C)-3') (158 µM), DNA primer 5'-d(GAC CAC GGC GC)-3') (158 µM), 1 mM ddCTP and 19 mM $MgCl_2$. Solutions were incubated at room temperature for 60 min and subsequently mixed in 1:1 ratio with the reservoir solution (100 mM Tris HCl, pH 7.5, 0.2 M magnesium formate, and 15 % PEG 8000). Crystals were produced by the hanging drop vapour diffusion method by equilibrating against 0.5 mL of the reservoir solution at 18 °C (Qiagen, EasyXtal 15-well tools). Crystal formation was observed after 1 day for DNA as template and after 3 days for the RNA template. Streak seeding led to the formation of crystals with improved diffraction characteristics in case of RNA as template. Crystals were flash-frozen in liquid nitrogen and were measured at 100 K with a wavelength of 1.00000 Å. Datasets were recorded on beamlines PXI and PXIII on a Pilatus 6M and 2M, respectively, at the SwissLightSource (SLS), Paul-Scherrer-Institut, Villigen, Switzerland. Data was processed and reduced using XDS.[22] The structure of RT-KTq 2_{DNA} was solved using difference Fourier methods. In case of RT-KTq 2_{RNA} molecular replacement (PHASER) was used to solve the phase problem.[23] Both structures were solved and refined using the PHENIX suite.[24] Manual refining and model rebuilding was performed using Coot.[25] Model quality was determined by the MolProbity web server.[26] Ramachandran statistics for RT-KTq 2_{DNA}: favored 97.61%, allowed 2.20 % and 0.18 % outlier. Statistics for RT-KTq 2_{RNA}: 98.53/1.47/0. Molecular graphics were drawn with Pymol.[27] Data collection and refinement statistics can be found in Table S2. In case of RT-KTq 2_{RNA} parts of the protein had to be retraced, as well as the hybrid duplex, neglecting the 2 outermost base pairs, which were not resolved.

RT-PCR with MS2 Bacteriophage RNA.[6] Reaction mixtures (20 µL) for real-time RT-PCR contained 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 0.1% Tween 20, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 100 nM of each primer (5'-d(ATC GCT CGA GAA CGC AAG TT)-3'; 5'-d(CG GAC TTC ATG CTG TCG GTG)-3'), 0.6x SYBRGreen I (Sigma-Aldrich), 5 nM of the respective purified DNA polymerase and 50 pg/µL MS2 RNA (Roche). First, reverse transcription was conducted using an initial denaturation step of 30 s at 95 °C, an

annealing step at 55 °C for 35 s and elongation for 7.5 min at 72 °C. After 1 min at 95 °C, 50 PCR cycles were performed with 30 s at 95 °C, 35 s at 55 °C and 40 s at 72 °C. Formation of double stranded DNA was visualized by binding of SYBRGreen I. Correct product formation was confirmed by agarose gel analysis.

Amplification of a 510 bp Fragment from MS2 RNA in RT-PCR. RT-PCR reactions contained 50 mM Tris HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 0.1 % Tween 20, 2.5 mM $MgCl_2$, 250 µM of each dNTP, 200 nM of each primer (5'-d(GAT CGC ATG CC TAG AGG CAC TTG CCT ACT ACG)-3'; 5'-d(GCT AAA GCT TCG GAC TTC ATG CTG TCG GTG ATT TC)-3'), 80 nM of the respective DNA polymerase and 350 pg/µl MS2 RNA (Roche). First, reverse transcription was conducted using an initial denaturation step of 60 s at 95 °C, an annealing step of 60 s at 65 °C and an elongation step of 30 min at 72 °C. 30 PCR cycles were subsequently performed with 60 s at 95 °C, 60 s at the respective annealing temperature and 90 s at 72 °C. Correct product formation was confirmed by agarose gel analysis.

Ultra-fast Two-step Cycling RT-PCR. Total RNA was extracted from Jurkat cells using the RNeasy Mini Plus Kit (Qiagen) and digested with DNase I (Thermo Scientific) according to the manufacturer's protocol. Reaction mixtures (10 µL) contained 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 0.1 % Tween 20, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 100 nM of each primer, 50 nM of DNA polymerase and 2.8 ng/µl total RNA. Cycling was performed with 30 s initial denaturation at 98 °C and 30 cycles of two step cycling with denaturation for 2 s at 92 °C and a combined annealing/extension for 15 s at the respective temperature. GAPDH (71 bp; 108 bp) transcripts were amplified at an annealing/extension temperature of 61 °C using primer forward (5'-d(GAA GGT GAA GGT CGG AGT CAA C)-3'; 5'-d(CGT CAA GGC TGA GAA CGG GA)-3') and reverse (5'-d(CAG AGT TAA AAG CAG CCC TGG T)-3'; 5'-d(ACG TAC TCA GCG CCA GCA TC)-3'), respectively. Beta actin (101 bp) and HPRT1 (111 bp) transcripts were amplified at an annealing/extension temperature of 72 °C and 63 °C respectively, using primer forward (5'-d(TGC CCT GGC ACC CAG CAC AA)-3') and reverse (5'-d(AGG TGG ACA GCG AGG CCA GGA)-3') for actin and primer forward (5'-d(TGC TGA GGA TTT GGA AAG GGT GTT)-3') and reverse (5'-d(AGG GCT ACA ATG TGA TGG CCT CC)-3') for HPRT1.

Real-time RT-PCR, Template Dilution Series. Total RNA was extracted from Jurkat cells using the RNeasy Mini Plus Kit (Qiagen) and digested with DNase I (Thermo Scientific) according to the manufacturer's protocol. Reaction mixtures of real-time RT-PCR experiments (20 µL) contained 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 0.1 % Tween 20, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 100 nM of each primer forward (5'-d

(CAC TCT TCC AGC CTT CCT TC)-3') and reverse (5'-d(GGA TGT CCA CGT CAC ACT TC)-3'), 1x SYBRGreen I, 50 nM of DNA polymerase and the respective concentration of total RNA. Template concentrations used in this experiment were in a range from 100 ng to 1 pg, ten-fold diluted in a step-wise manner. Cycling was performed with 60 s initial denaturation at 95 °C and 37 cycles of two-step cycling with denaturation for 15 s at 95 °C and combined annealing/extension for 30 s at 72 °C. Formation of double stranded DNA was visualized by binding of SYBRGreen I.

Sensitivity of RNA detection with commercially available kits in RT-PCR. RT-experiments examining the sensitivity of RT-KTq 2 compared to Titan One Kit (Roche) and Superscript III One-Step RT-PCR System (Invitrogen) were performed for KTq DNA polymerase as described in Section "Real-time RT-PCR, template dilution series" without the addition of SYBRGreen I and template concentrations in a range from 1 ng to 1 pg, ten-fold diluted in a step-wise manner. Reactions with the commercially available kits were performed according to the manual with the same set-up of primers and template. Cycle conditions for the Titan One Kit comprised a reverse transcription step at 55 °C for 30 min. Next, initial denaturation at 94 °C for 2 min was performed preceding 10 PCR cycles with denaturation for 30 s at 94 °C, annealing for 30 s at 65 °C and elongation for 45 s at 68 °C. Finally, an additional 25 PCR cycles with denaturation for 30 s at 94 °C, annealing for 30 s at 65 °C and an increased elongation time of 60 s at 68 °C were conducted. At the end a final elongation step of 4 min at 68 °C was performed. Cycle conditions for the Superscript III One-Step RT-PCR System (Invitrogen) comprised a reverse transcription step at 60 °C for 15 min. Next, initial denaturation at 94 °C for 2 min was performed preceding 37 PCR cycles with denaturation for 15 s at 94 °C, annealing for 30 s at 65 °C and elongation for 30 s at 68 °C. Correct product formation was confirmed by agarose gel analysis.

Taq Man Assay, Detection of Influenza Virus A. RNA extracts of respiratory swab samples were from patients known to be influenza A positive. All samples were previously analyzed with the commercial RIDA®GENE Flu assay (R-Biopharm AG, Darmstadt, Germany) which detects influenza A incl. H1N1 variant. The analytical sensitivity is given with 50 copies per mL by the manufacturer. Specimens which yielded ct-values in a LightCycler 2.0 instrument of 35 or less were regarded as being positive for the respective influenza virus, ct values of >35 and <40 were interpreted as "weak positive". RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen) and digested with DNase I (ThermoScientific) according to the manufacturer's protocol. Primers for influenza A virus detection (recommended by the WHO in "WHO information for molecular diagnosis of influenza virus in humans", August 2011) target the RNA coding for the matrix protein (5'-d(CCM AGG TCG AAA CGT AYG TTC TCT CTA TC)-3'; 5'-d(TGA CAG RAT YGG TCT TGT CTT TAG CCA YTC CA)-3'). TaqMan probe for influenza A detection consisted of an oligonucleotide 5'-d(ATY TCG GCT TTG AGG GGG CCT G)-3' with a 5' reporter dye 6-carboxyfluorescein (FAM) and a 3' minor groove binder (MGB). Reactions mixtures (10 µL) for influenza A detection contained 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 0.1 % Tween 20, 7.5 mM $MgCl_2$, 500 µM of each dNTP, 0.8 M betaine, 600 nM of each primer, 100 nM of the respective TaqMan probe, 2.5 µL undiluted isolated RNA of influenza A positive samples, 50 nM of RT-KTq 2 polymerase and 10 nM Taq wild-type. To increase reaction specificity an aptamer (5'-d(TTC TCG GTT GGT CTC TGG CGG AGC AAG ACC AGA CAA TGT ACA GTA TT G GCC TGA TCT TGT GTA TGA TTC GCT TTT CCC)-3') with the ability to inhibit Taq polymerase at low temperatures was added to the reaction.[28] Cycling was performed with 15 s initial denaturation at 95 °C and 46 cycles of two step cycling with denaturation for 3 s at 95 °C and combined annealing/extension for 45 s at 63.5 °C.

Supplementary Figures

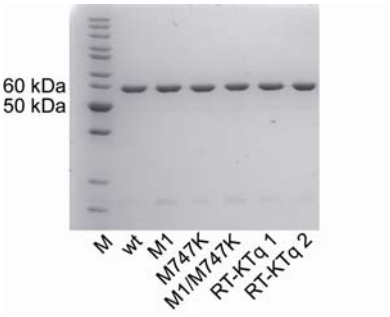

Figure S1. SDS-PAGE analysis of purified KTq wild-type (wt) and variants.

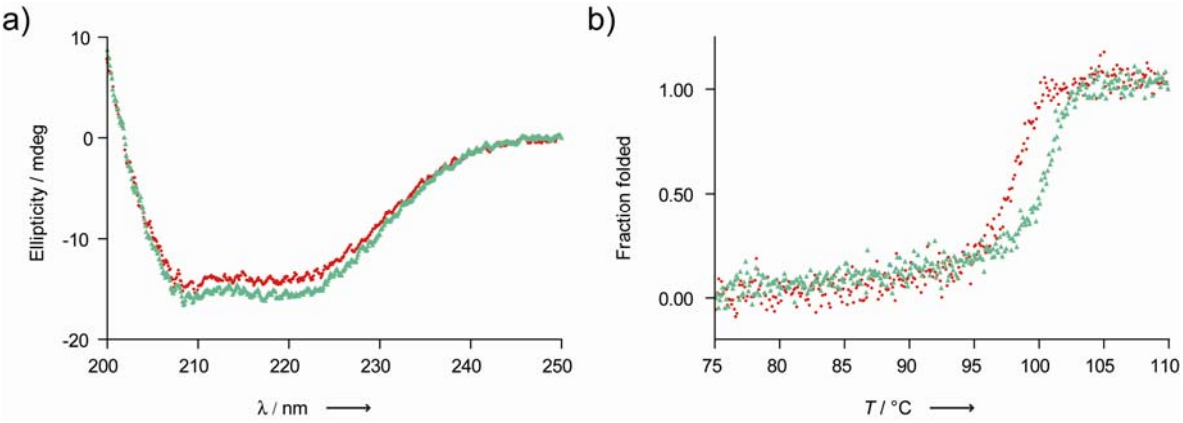

Figure S2. Stability of KTq M1/M747K and RT-KTq 2. a) CD-spectra of KTq M1/M747K and RT-KTq 2 are shown in red and green, respectively. b) Thermal denaturation of KTq M1/M747K and RT-KTq 2 was measured following the ellipticity at 209 nm. Melting temperatures were determined in two separate experiments for KTq M1/M747K (T_M 97.9 ± 0.1 °C) and RT-KTq 2 (T_M 100.1 ± 0.1 °C).

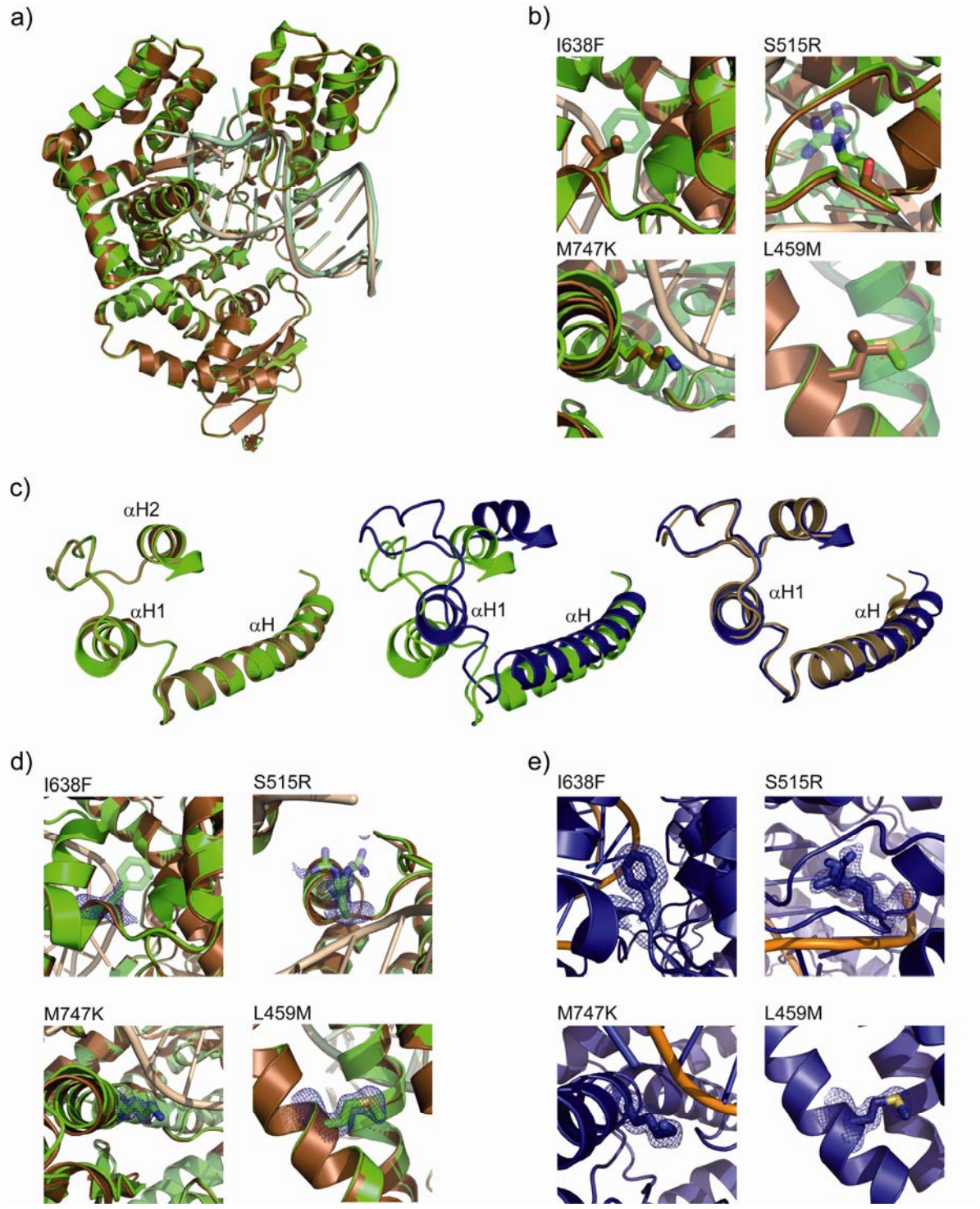

Figure S3. Structural details of KTq wild-type, RT-KTq 2_{DNA} and RT-KTq $2_{RNA.}$ a) Structure of KTq wild-type (sand, PDB 3RTV) superimposed with RT-KTq 2_{DNA} (green) bound to DNA-duplex. Mutation sites are highlighted in b). c) Structural relocation of αH, αH1 and αH2 in the thumb domain. Overlay of protein structure of KTq wild-type (PDB 3RTV, sand) and RT-KTq 2_{DNA} (green) shows no relocation of the motif (left). Superimposition of protein structure of RT-KTq 2_{DNA} (green) and RT-KTq 2_{RNA} (blue) reveals shift in αH, αH1 and αH2 (middle). Superimposition of the motif alone reveals the origin of the shift. Deviation starts in the N-terminal portion of αH containing the L459M mutation (right). d)-e) Simulated annealing omit maps (σ level of 3) showing mutation sites of RT-KTq 2. d) Mutation sites in RT-KTq 2_{DNA} (green) superimposed with KTq wild-type (PDB 3RTV, sand). e) Mutation sites in RT-KTq 2_{RNA}.

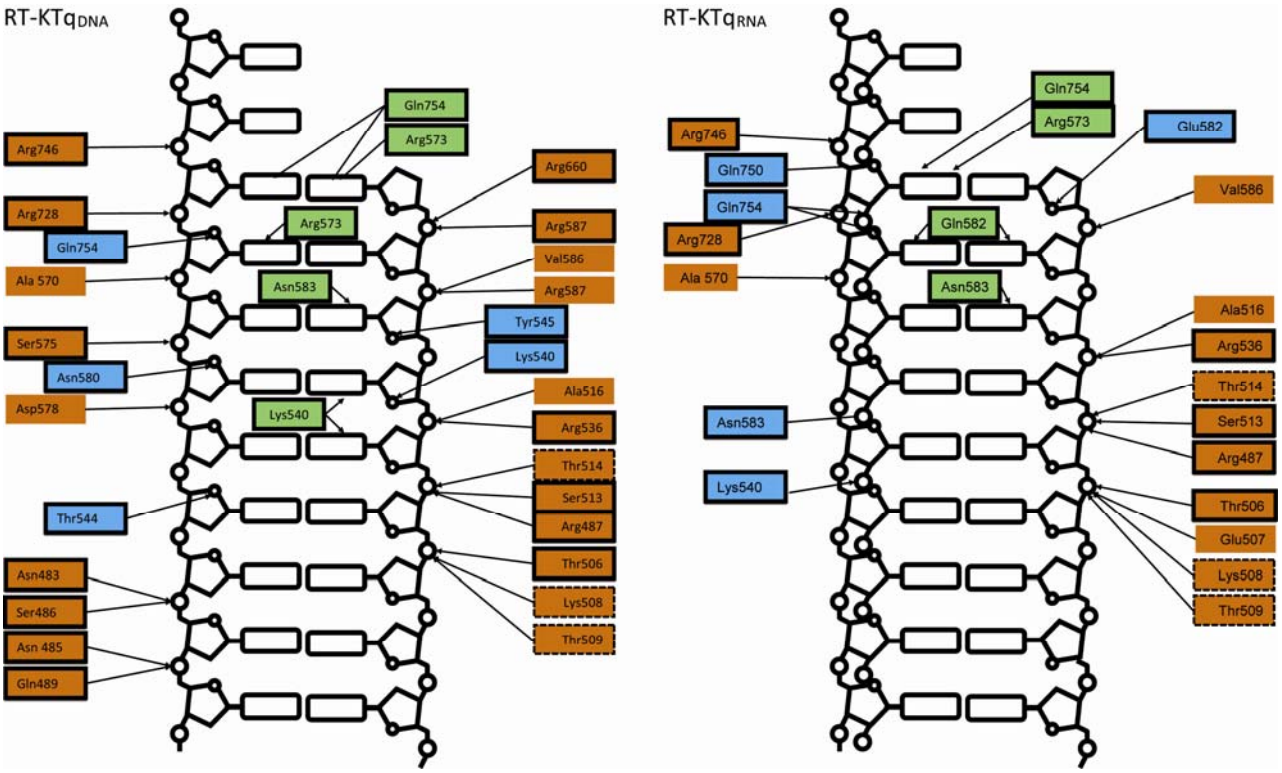

Figure S4. Interaction map of RT-KTq 2 (closed ternary complex) with DNA duplex (left) and RT-KTq 2 with DNA/RNA hybrid (right). Only direct contacts up to a distance of 3.6 Å are shown. Side chain interactions are marked with a solid lining, contacts with the protein backbone are shown without lining and residues where both interactions are found are shown with dashed lining. Interactions to the phosphate backbone, sugar oxygen or nucleobase are shown in orange, blue and green, respectively.

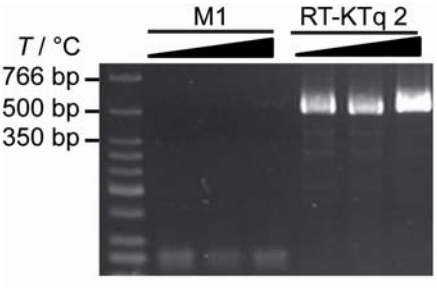

Figure S5. RT-PCR amplifying a 510 bp fragment from MS2 bacteriophage RNA. The annealing step was performed at 65, 68 and 70 °C, respectively (T_{Anneal}).

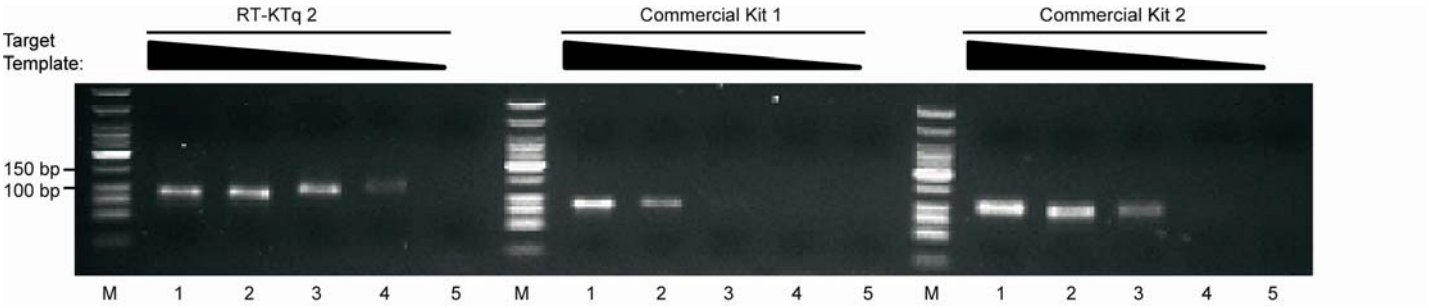

Figure S6. Comparison of sensitivity of RT-KTq 2 with commercially available Superscript III One-Step RT-PCR System (Invitrogen) (Commercial Kit 1) and Titan One Kit (Roche; Commercial Kit 2). Both kits comprise an enzyme blend of reverse transcriptase and thermostable DNA polymerase. The experiments with the commercially available kits were performed according to the manual of the manufacturer. A 90 bp fragment from human beta actin was amplified from total RNA (Jurkat cells) with decreasing template amounts of 1000 pg (lane 1), 100 pg (lane 2), 10 pg (lane 3), 1 pg (lane 4) and without RNA as control (lane 5). Product formation was analyzed on a 2.5 % agarose gel. M: Marker.

Supplementary Tables

Table S1. Sugar pucker conformations of primer and template nucleotides.

Primer

Base	v0	v1	v2	v3	v4	P	vmax	χ	γ	type
dC	11.21	-23.74	26.61	-20.82	6.21	**354.47**	26.73	-163.74	160.71	C2'-exo
dC	-2.24	-21.63	35.56	-37.7	25.45	**21.8**	38.3	-161.6	45.25	C3'-endo
dA	-1.58	-23.29	37.72	-39.59	26.13	**20.76**	40.34	-166.82	54.64	C3'-endo
dC	1.63	-25.09	37.5	-37.48	22.83	**16.23**	39.05	-160.48	50.12	C3'-endo
dG	-18.04	9.46	2.04	-12.48	19.07	**83.93**	19.29	-139.13	51.44	O4'-endo
dG	-19.66	34.04	-34.85	24.39	-3.18	**166.31**	35.87	-112.52	50.37	C2'-endo
dC	-43.81	38.72	-19.84	-4.78	30.39	**117.42**	43.08	-125.91	48.32	C1'-exo
dG	-32.22	46.09	-42.2	25.4	4	**156.34**	46.07	-113.74	28.87	C2'-endo
dC	1.61	-26.4	40.84	-41.7	24.75	**17**	42.71	-156.56	174.77	C3'-endo
ddC	1.31	-19.96	30.01	-29.15	17.36	**15.28**	31.11	-149.56	51.87	C3'-endo

Template

Base	v0	v1	v2	v3	v4	P	vmax	χ	γ	type
A	-19.93	35.34	-36.3	25.8	-3.91	167.11	37.24	13.35	57.75	C2'-endo
A	-22.15	37.44	-37.52	25.76	-2.48	164.81	38.88	-142.24	-172.22	C2'-endo
A	-26.09	40.25	-38.17	24.12	1.08	159.76	40.68	123.09	-147.65	C2'-endo
G	7.21	-28	36.98	-33.97	16.96	**7.86**	37.33	-176.53	-168.16	C3'-endo
G	6.08	-27.45	37.18	-34.67	18.05	**9.52**	37.7	-162.19	50.47	C3'-endo
G	4.9	-26.87	37.58	-35.77	19.45	**11.46**	38.35	-156.88	51.82	C3'-endo
C	1.7	-25.66	38.57	-38.73	23.39	**16.32**	40.19	-158.8	57.18	C3'-endo
G	10.49	-27.7	33.52	-28.57	11.46	**1.02**	33.53	-173.83	-167.56	C3'-endo
C	2.5	-24.5	35.91	-35.48	20.85	**14.86**	37.16	-159.5	50.84	C3'-endo
C	1.33	-23.15	34.88	-35.07	21.3	**16.55**	36.39	-154.96	56.9	C3'-endo
G	2.3	-24.54	36.27	-35.9	21.2	**15.16**	37.58	-159.16	50.24	C3'-endo
U	1.44	-23.81	35.94	-36.13	21.87	**16.49**	37.48	-160.42	48.39	C3'-endo
G	2.15	-23.4	34.51	-34.25	20.3	**15.27**	35.78	-179.1	174.01	C3'-endo
G	4.94	-26.31	36.5	-34.66	18.77	**11.17**	37.2	179.37	159.34	C3'-endo

***Table S1*.** The sugar puckers in the primer nucleotides show several different conformations ranging from C3'-endo conformations for sugar residues free from enzyme interactions to C2'-endo, C1'-exo and O4'-endo. The sugar puckers in the template nucleotides adopt RNA-typical A-form conformation. The first three nucleotides in the template show a C2'-endo conformation, but due to low resolution of these residues we cannot make any predictions about the sugar conformations of these residues. P-values for the first three bases of the template are therefore not highlighted in bold. The two outermost base pairs are not listed, as they were not resolved in **RT-KTq 2$_{RNA}$**.

Table S2. Data collection and refinement statistics (Molecular replacement)

	RT-KTq 2_{DNA} PDB ID 4BWJ	RT-KTq 2_{RNA} PDB ID 4BWM
Data collection		
Space group	152 ($P3_121$)	18 ($P2_12_12$)
Cell dimensions		
a, *b*, *c* (Å)	a=b=108,.43 c=89.81	143.19 86.28 63.09
α, β, γ (°)	90, 90, 120	90, 90, 90
Resolution (Å)	46.83(1.64)-1.55	47.985(1.85)-1.749
R_{meas}	6.3 (112.5)	8.3 (111.5)
$I/\sigma I$	15.56 (1.64)	13.73(1.70)
Completeness (%)	99.7 (98.0)	99.9 (99.4)
Redundancy	9.6 (8.6)	6.59 (6.62)
Refinement		
Resolution (Å)	47.99-1.55	46.83-1.75
No. reflections	87950	79632
R_{work}/ R_{free}	15.9/20.1	16.8/20.8
No. atoms (H/noH)		
Protein	8765/4345	-/4345
Ligand/ion	906/569	-/548
Water	476/476	-/624
B-factors		
Protein	43.80	33.97
Nucleic acids	36.52	71.52
Water	42.29	43.59
R.m.s deviations		
Bond lengths (Å)	0.011	0.007
Bond angles (°)	1.504	1.089

*Highest resolution shell is shown in parenthesis.

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