

REVERSE TRANSCRIPTASE MUTANTS WITH INCREASED ACTIVITY AND THERMOSTABILITY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 63/054,228 filed July 20, 2020. The above listed application is incorporated by reference herein in its entirety for all purposes.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically as a text file in ASCII format and is hereby incorporated by reference in its entirety. The name of the ASCII text file is “20-1076-WO_Sequence-Listing_ST25_FINAL.txt”, was created on July 19, 2021, and is 492 kilobytes in size.

FIELD OF THE DISCLOSURE

The disclosure relates to Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutants. The disclosure also relates to suitable amino acid positions in MMLV RTase for mutagenesis and methods for using MMLV RTase mutants to synthesize cDNA from RNA templates.

BACKGROUND

Reverse transcriptase (RTase) enzymes have revolutionized molecular biology. RTase is a critical component of the reverse transcription polymerase chain reaction (RT-PCR) allowing the production of complementary DNA (cDNA) from RNA. The cDNA produced in reverse transcription reactions can be used in a wide range of downstream applications, including quantitative PCR, gene expression analysis, isolated RNA sequencing, gene cloning, and cDNA library creation.

RTases, first derived from retroviruses, facilitate the reverse transcription of RNA into cDNA by utilizing RNA-dependent polymerase and RNase H, a non-sequence-specific endonuclease enzyme that catalyzes cleavage of RNA in an RNA/DNA duplex. This results in virus replication and integration of the viral sequence into host DNA thereby allowing for the proliferation of the virus along with host DNA. Within the laboratory setting, RTases from Moloney murine leukemia virus (MMLV), avian myeloblastosis virus (AMV), and

human immunodeficiency virus type 1 (HIV-1) are the most commonly used RTase for cDNA synthesis.

RTases for research applications are often mutated multi-generational MMLV and AMV RTases that have been optimized for laboratory procedures. Mutations in the RTases alter properties of the enzymes, including thermostability, RTase activity, 5' mRNA coverage, and RNase H activity.

AMV RTases are thermostable and less sensitive to thermal degradation than MMLV RTase and are preferred for RNA having a strong secondary structure. In addition, AMV RTases are often suitable for use with RNA molecules that are five kilobases or longer because of the heat stability of AMV RTases. However, the high temperatures required to resolve strong secondary structures or long RNA strands can negatively impact RNA integrity and fidelity of transcription. AMV also possess an intrinsic RNase activity that degrades RNA in an RNA/DNA hybrid, which can result in reduced total cDNA and reduced full-length cDNA yield.

MMLV RTase is characterized by low RNase H activity and a higher fidelity as compared to AMV RTase. The reduced RNase H activity allows MMLV RTases to be used for the reverse transcription of long RNAs (>5kb). However, the RNase H activity of MMLV RTase limits the efficiency of synthesizing long cDNA *in vitro*. Mutations in MMLV RTase have been introduced to reduce RNase H activity. In addition, because the optimal temperature for MMLV RTase activity is ~37°C, the enzyme lacks the ability to effectively reverse transcribe RNAs with strong secondary structures. The use of MMLV RTase at elevated temperatures can compromise cDNA length and yield as a result of lower enzyme activity. MMLV RTase mutants that substitute Mn²⁺ for Mg²⁺ in the reaction mixture attempt to overcome these limitations, but are characterized by inefficiency and error.

Thus, despite the unique properties of AMV and MMLV RTases, there exists a need for an RTase that combines the beneficial attributes of AMV and MMLV RTases. Consistent with this, the present application discloses MMLV RTase mutants, isolated through rational mutagenesis of MMLV RTase, that exhibit increased RTase activity and thermostability as compared to RTases, including RNase H minus constructs, that are currently available in the art.

SUMMARY

The disclosure provides Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutants. The disclosure also provides suitable amino acid positions in MMLV RTase for mutagenesis and methods and kits for using MMLV RTase mutants to synthesize cDNA from RNA templates.

One aspect of the disclosure provides an isolated Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutant comprising the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least one amino acid substitution that is: (a) an isoleucine to arginine, lysine or methionine substitution at position 61 (I61R, I61K or I61M); (b) a glutamine to arginine, lysine or isoleucine substitution at position 68 (Q68R, Q68K or Q68I); (c) a glutamine to arginine, histidine or isoleucine substitution at position 79 (Q79R, Q79H or Q79I); (d) a leucine to arginine, lysine or asparagine substitution at position 99 (L99R, L99K or L99N); (e) a glutamic acid to aspartic acid, methionine or typtophan substitution at position 282 (E282D, E282M or E282W); and/or (f) an arginine to alanine substitution at position 298 (R298A).

Another aspect of the disclosure provides an isolated Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutant comprising the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least two amino acid substitutions that are: (a) an isoleucine to arginine substitution at position 61 (I61R); (b) a glutamine to arginine substitution at position 68 (Q68R); (c) a glutamine to arginine substitution at position 79 (Q79R); (d) a leucine to arginine substitution at position 99 (L99R); (e) a glutamic acid to aspartic acid substitution at position 282 (E282D); and/or (f) an arginine to alanine substitution at position 298 (R298A): (a) an isoleucine to arginine substitution at position 61 and a glutamic acid to aspartic acid substitution at position 282 (I61R/E282D); (b) a leucine to arginine at substitution position 99 and a glutamic acid to aspartic acid substitution at position 282 (L99R/E282D); (c) a glutamine to arginine substitution at position 68 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/E282D); (d) a glutamine to arginine substitution at position 79 and a glutamic acid to aspartic acid substitution at position 282 (Q79R/E282D); (e) a glutamic acid to aspartic acid substitution at position 282 and an arginine to alanine substitution at position 298 (E282D/R298A); (f) an isoleucine to arginine substitution at position 61 and a leucine to arginine substitution at position 99 (I61R/L99R); (g) an isoleucine to arginine substitution at position 61 and a glutamine to arginine substitution at position 68 (I61R/Q68R); (h) an isoleucine to arginine substitution at position 61 and a glutamine to arginine substitution at position 79 (I61R/Q79R); (i) an isoleucine to arginine

substitution at position 61 and an arginine to alanine substitution at position 298 (I61R/R298A); (j) a glutamine to arginine substitution at position 68 and a leucine to arginine substitution at position 99 (Q68R/L99R); (k) a glutamine to arginine substitution at position 79 and a leucine to arginine substitution at position 99 (Q79R/L99R); (l) a leucine to arginine substitution at position 99 and an arginine to alanine substitution at position 298 (L99R/R298A); (m) a glutamine to arginine substitution at position 68 and a glutamine to arginine substitution at position 79 (Q68R/Q79R); (n) a glutamine to arginine substitution at position 68 and an arginine to alanine substitution at position 298 (Q68R/R298A); or (o) a glutamine to arginine substitution at position 79 and an arginine to alanine substitution at position 298 (Q79R/R298A).

Another aspect of the disclosure provides an isolated Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutant comprising the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least three amino acid substitutions that are: (a) a glutamine to arginine substitution at position 68 (Q68R); (b) a glutamine to arginine substitution at position 79 (Q79R); (c) a leucine to arginine substitution at position 99 (L99R); and/or (d) a glutamic acid to aspartic acid substitution at position 282 (E282D): (a) a glutamine to arginine substitution at position 68, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/L99R/E282D); (b) a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q79R/L99R/E282D); (c) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 68 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79R/E282D); or (d) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 68 and a leucine to arginine substitution at position 99 (Q68R/Q79R/L99R).

Another aspect of the disclosure provides an isolated Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutant comprising the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least four amino acid substitutions that are: (a) a glutamine to arginine, lysine or isoleucine substitution at position 68 (Q68R, Q68K or Q68I); (b) a glutamine to arginine, histidine or isoleucine substitution at position 79 (Q79R, Q79H or Q79I); (c) a leucine to arginine, lysine or asparagine substitution at position 99 (L99R, L99K or L99N); (d) a glutamic acid to aspartic acid, methionine or typtophan substitution at position 282 (E282D, E282M or E282W); (a) a glutamine to arginine substitution at position 68, a glutamine to arginine

substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79R/L99R/E282D); (b) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to lysine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79R/L99K/E282D); (c) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to asparagine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79R/L99N/E282D); (d) a glutamine to isoleucine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68I/Q79R/L99R/E282D); (e) a glutamine to lysine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68K/Q79R/L99R/E282D); (f) a glutamine to arginine substitution at position 68, a glutamine to histidine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79H/L99R/E282D); (g) a glutamine to arginine substitution at position 68, a glutamine to isoleucine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79I/L99R/E282D); (h) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to methionine substitution at position 282 (Q68R/Q79R/L99R/E282M); (i) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to tryptophan substitution at position 282 (Q68R/Q79R/L99R/E282W); (j) a glutamine to isoleucine substitution at position 68, a glutamine to histidine substitution at position 79, a leucine to lysine substitution at position 99 and a glutamic acid to methionine substitution at position 282 (Q68I/Q79H/L99K/E282M);;

Another aspect of the disclosure provides an isolated Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutant comprising the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least five amino acid substitutions that are: (a) an isoleucine to lysine or methionine substitution at position 61 (I61K or I61M); (b) a glutamine to arginine or isoleucine substitution at position 68 (Q68R or Q68I); (c) a glutamine to arginine or histidine

substitution at position 79 (Q79R or Q79H); (d) a leucine to arginine or lysine substitution at position 99 (L99R or L99K); (e) a glutamic acid to aspartic acid or methionine substitution at position 282 (E282D or E282M): (a) an isoleucine to lysine substitution at position 61, a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (I61K/Q68R/Q79R/L99R/E282D); (b) an isoleucine to methionine substitution at position 61, a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (I61M/Q68R/Q79R/L99R/E282D); or (c) an isoleucine to methionine substitution at position 61, a glutamine to isoleucine substitution at position 68, a glutamine to histidine substitution at position 79, a leucine to lysine substitution at position 99 and a glutamic acid to methionine substitution at position 282 (I61M/Q68IR/Q79H/L99K/E282M).

Another aspect of the disclosure provides an isolated Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutant comprising the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least five or more amino acid substitutions that are : (a) a glutamine to arginine, lysine or isoleucine substitution at position 68 (Q68R, Q68K or Q68I); (b) a glutamine to arginine, histidine or isoleucine substitution at position 79 (Q79R, Q79H or Q79I); (c) a leucine to arginine, lysine or asparagine substitution at position 99 (L99R, L99K or L99N); (d) a glutamic acid to aspartic acid, methionine or typtophan substitution at position 282 (E282D, E282M or E282W); (e) a glutamine to glutamic acid substitution at position 299; (f) threonine to glutamic acid substitiuon at position 332; (g) valine to arginine substitution at position 433; (h) isoleucine to glutamic acid substitution at position 593; (a) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282, a glutamine to glutamic acid subsitution at position 299, a valine to arginine substitution at position 433 and a isoleucine to glutamic acid at position 593 (Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E); (b) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to argine substitiuon at postion 82, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282, a glutamine to glutamic acid subsitution at position 299, a valine to arginine substution at position 433 and a isoleucine to glutamic acid at position 593 (Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E); (c) a glutamine to arginine

substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 82, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282, a glutamine to glutamic acid substitution at position 299, a threonine to glutamic acid substitution at position 332, and a isoleucine to glutamic acid at position 593

(Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E); (d) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 82, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282, a glutamine to glutamic acid substitution at position 299, a threonine to glutamic acid substitution at position 332, a valine to arginine substitution at position 433, and a isoleucine to glutamic acid at position 593 (Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E)

Another aspect of the disclosure provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding an MMLV RTase mutant of the disclosure.

Other aspects of the disclosure provide a composition or a kit comprising an MMLV RTase mutant of the disclosure.

Other aspects of the disclosure provide methods for synthesizing complementary deoxyribonucleic acid (cDNA) or methods for performing reverse transcription-polymerase chain reaction (RT-PCR) using an MMLV RTase mutant of the disclosure.

Specific embodiments of the disclosure will become evident from the following more detailed description and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1C are schematics showing reverse transcriptase mutagenesis selection by rational design. Amino acid positions for mutagenesis were chosen at the substrate binding site (Figures 1A and 1B) or near the substrate binding site (Figure 1C).

Figure 2 shows Western blot analysis of test induction results in BL21(DE3) cells for MMLV RT in TB medium. Lane 1 – Precision Plus Protein Unstained Standards (Bio Rad, Cat #161-0363), Lane 2 – Time = 0 hour, Lane 3 – Time = 3 hours after induction at 37°C, Lane 4 – Time = 0 hour, Lane 5 – Time = 21 hours after induction at 18°C.

DETAILED DESCRIPTION

The disclosure relates to Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutants. The disclosure also relates to suitable amino acid positions in MMLV RTase for mutagenesis and methods and kits for using MMLV RTase mutants to synthesize cDNA from RNA templates.

The MMLV RTase mutants of the disclosure, which have been identified and isolated, at least in part, through rational mutagenesis of a base construct of MMLV RTase, were found to have increased RTase activity and thermostability as compared to wild-type MMLV RTase and certain MMLV RTase mutants, including RNase H minus RTases, that are currently available in the art.

Reference will now be made in detail to exemplary embodiments of the claimed invention. While the claimed invention will be described in conjunction with the exemplary embodiments, it will be understood that it is not intended to limit the claimed invention to those embodiments. To the contrary, it is intended to cover alternatives, modifications, and equivalents, as may be included within the spirit and scope of the claimed invention, as defined by the appended claims.

Those of ordinary skill in the art may make modifications and variations to the embodiments described herein without departing from the spirit or scope of the claimed invention. In addition, although certain methods and materials are described herein, other methods and materials that are similar or equivalent to those described herein can also be used to practice the claimed invention.

In addition, any of the compositions or methods provided, disclosed, or described herein can be combined with one or more of any of the other compositions and methods provided, disclosed, or described herein.

1. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which the claimed invention belongs. The terminology used herein is for describing particular embodiments only and is not intended to be limiting of the claimed invention. All technical and scientific terms used herein have the same meaning.

The following references provide those of skill in the art with a general understanding of many of the terms used herein (unless defined otherwise herein): Singleton *et al.*, Dictionary of Microbiology and Molecular Biology, 3rd ed. (Wiley, 2006); Walker, The Cambridge Dictionary of Science and Technology (Cambridge University Press, 1990);

Rieger *et al.*, Glossary of Genetics: Classical and Molecular, 5th ed. (Springer Verlag, 1991); and Hale *et al.*, Harper Collins Dictionary of Biology (HarperCollins Publishers, 1991). Generally, the procedures or methods described herein and the like are common methods used in the art. Such standard techniques can be found in reference manuals such as, for example, Green *et al.*, Molecular Cloning: A Laboratory Manual, 4th ed. (Cold Spring Harbor Laboratory Press, 2012), and Ausubel, Current Protocols in Molecular Biology (John Wiley & Sons Inc., 2004).

The following terms may have meanings ascribed to them below, unless specified otherwise. However, it should be understood that other meanings known or understood by those having ordinary skill in the art are also possible, and within the scope of the claimed invention. All publications, patent applications, patents, and other references mentioned or discussed herein are expressly incorporated by reference in their entireties. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

As used herein, the singular forms "a," "and," and "the" include plural references, unless the context clearly dictates otherwise.

As used herein, the term "or" means, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

As used herein, the term "including" means, and is used interchangeably with, the phrase "including but not limited to."

As used herein, the term "such as" means, and is used interchangeably with, the phrase "such as, for example" or "such as but not limited."

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example, within two standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1 %, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein can be modified by the term about.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

As used herein, the terms "nucleic acid molecule" and "polynucleotide" refer to a polymer or large biomolecule comprised of nucleotides. The term "nucleic acid" includes

deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and analogs thereof. Non-limiting examples of nucleic acid molecules include DNA (*e.g.*, genomic DNA, cDNA), RNA molecules (*e.g.*, mRNA, rRNA, cRNA, tRNA), and chimeras thereof. A nucleic acid molecule can be obtained by cloning techniques or synthesized, using techniques that are known to those of skill in the art. DNA can be double-stranded or single-stranded (coding strand or non-coding strand, *i.e.*, antisense). A nucleic acid backbone may comprise a variety of linkages known in the art, including one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds (referred to as "peptide nucleic acids" (PNA)), phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar moieties of the nucleic acid may be ribose or deoxyribose, or similar compounds having known substitutions, for example, 2' methoxy substitutions (containing a 2'-O-methylribofuranosyl moiety) and/or 2' halide substitutions. Nitrogenous bases may be conventional bases (adenine (A), guanine (G), thymine (T), cytosine (C), and uracil (U)), known analogs thereof (*e.g.*, inosine), known derivatives of purine or pyrimidine bases, or "abasic" residues in which the backbone includes no nitrogenous base for one or more residues. A nucleic acid may comprise only conventional sugars, bases, and linkages, as found in RNA and DNA, or may include both conventional components and substitutions (*e.g.*, conventional bases linked via a methoxy backbone, or a nucleic acid including conventional bases and one or more base analogs). An "isolated nucleic acid molecule," as is generally understood by those of skill in the art and as used herein, refers to a polymer of nucleotides, and includes but is not limited to DNA and RNA.

As used herein, the term "probe" refers to a nucleic acid oligonucleotide that hybridizes specifically to a target sequence in a nucleic acid or its complement, under conditions that promote hybridization, thereby allowing detection of the target sequence or its amplified nucleic acid. Detection may either be direct (*i.e.*, resulting from a probe hybridizing directly to the target or amplified sequence) or indirect (*i.e.*, resulting from a probe hybridizing to an intermediate molecular structure that links the probe to the target or amplified sequence). A probe's "target" generally refers to a sequence within an amplified nucleic acid sequence (*i.e.*, a subset of the amplified sequence) that hybridizes specifically to at least a portion of the probe sequence by standard hydrogen bonding or "base pairing." Sequences that are "sufficiently complementary" allow stable hybridization of a probe sequence to a target sequence, even if the two sequences are not completely complementary. A probe may be labeled or unlabeled. A probe can be produced by molecular cloning of a

specific DNA sequence or it can be synthesized. Probes for use in the methods disclosed herein can be readily designed and used by those of skill in the art.

As used herein, the term "primer" refers to a nucleic acid oligonucleotide that hybridizes specifically to a target sequence in a nucleic acid or its complement, and which is capable of priming the synthesis of a nascent nucleic acid in a template-dependent process. Primers may be provided in double-stranded or single-stranded form. Primers for use in the methods disclosed herein can be readily designed and used by those of skill in the art.

Probes or primers for use in the methods disclosed herein may be of any suitable length, depending on the particular assay format and the particular needs and targeted sequences employed. For example, the probes or primers for use in the methods disclosed herein are at least 10 nucleotides in length, or at least 15, 20, 25, 30, or more than 30 nucleotides in length, and they may be adapted to be especially suited for a chosen nucleic acid amplification system and/or hybridization system used. Longer probes and primers are also within the scope of the disclosure.

A "transcribed polynucleotide" or "nucleotide transcript" is a polynucleotide (*e.g.*, mRNA, hnRNA, cDNA, or analog of such RNA or cDNA) that is complementary to or having a high percentage of identity (*e.g.*, at least 80% identity) with all or a portion of a mature mRNA made by transcription of a marker of the disclosure and normal post-transcriptional processing (*e.g.*, splicing), if any, of the RNA transcript, and reverse transcription of the RNA transcript.

As used herein, the terms "reverse transcriptase," "RTase," or "RT" refer to an enzyme that is used to generate complementary (cDNA) from an RNA template in a process known as "reverse transcription." The term reverse transcriptase, as used herein, also refers to any enzyme that exhibits reverse transcription activity. Reverse transcriptases can be derived from a variety of sources including but not limited to viruses including retroviruses and DNA polymerases exhibiting transcriptase activity. Such retroviruses include but are not limited to Moloney murine leukemia virus (MMLV), avian myeloblastosis virus (AMV), and human immunodeficiency virus (HIV).

Reverse transcriptase activity can be measured by incubating an RTase in a buffer containing an RNA template and deoxynucleotides. One of skill in the art will recognize that a wide range of conditions can be used to perform reverse transcription reactions and multiple methods exist for measuring the quantity of cDNA produced during reverse transcription.

Reverse transcriptases of the disclosure include reverse transcriptases having one or a combination of the properties described herein. Such properties include but are not limited to

increased activity, enhanced DNA synthesis, enhanced stability or enhanced thermostability, reduced or eliminated RNase H activity, reduced terminal deoxynucleotidyl transferase activity, increased accuracy or increased fidelity, increased specificity, or altered half-life, for example when compared to a base construct. As used herein, the term "base construct" refers to the initial RTase from which the RTase mutants of the disclosure are prepared (*e.g.* for example a wild-type RTase or a modified wild-type RTase).

As used herein, the terms "accuracy" and "fidelity" are used interchangeably and refer to ability of an RTase to accurately replicate a desired template; *i.e.*, the ability of the RTase to accurately perform cDNA synthesis in a reverse transcription reaction. The "fidelity" or "accuracy" of a reverse transcriptase can be assessed by determining the frequency of incorrect nucleotide incorporation into the synthesized cDNA molecule, which may be referred to as the enzyme's error rate. As used herein, the term "increased fidelity" refers to RTase mutants of the disclosure that exhibit an error rate lower than that of the base construct. For example, the RTase mutants as disclosed herein can exhibit an error rate that is 10 %, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or 200% lower than, or at least 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold, or more than 10-fold lower than the error rate of the RTase base construct...

As used herein, the term "specificity" refers to a decrease in mis-priming by an RTase during cDNA synthesis. An RTase mutant's specificity can be assessed by performing a reverse transcription reaction at a particular temperature, including higher temperatures, and comparing the amount of mis-priming in that reaction with the amount of mis-priming in a reaction performed with the wild-type RTase (or the RTase base construct) under identical conditions.

As used herein with respect to the RTase molecules of the disclosure, the terms "stable" and "thermostable" are used interchangeably and refer to an enzyme that is resistant to heat inactivation and remains active at temperatures in excess of 37°C (*e.g.*, 38°C, 39°C, 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 70°C, or higher temperatures). For example, in one embodiment the disclosure provides an RTase mutant having activity with a longer half-life than that of the base construct RTase at an elevated temperature. Thus, RTase mutants with "enhanced thermostability" can refer to RTase mutants of the disclosure that exhibit an increase in thermostability at temperatures of about 50°C up to about 90°C as compared to the base construct RTase. In some embodiments, the thermostability of the RTase mutant is at least 1.5 fold or greater as compared to the

thermostability of the base construct RTase. Comparisons of cDNA produced by a base construct and RTase mutant are compared using identical reaction conditions for the base construct and RTase mutant reactions. Reaction conditions can include but are not limited to salt concentration, buffer concentration, pH, divalent metal ion concentration, temperature, nucleoside triphosphate concentration, template concentration, RTase concentration, primer concentration, time, and in one-step PCR, the quantitative PCR primer and probe concentrations.

As used herein, the term "enhanced DNA synthesis" refers to an RTase enzyme that produces more DNA (e.g. cDNA) than the base RTase construct. In some embodiments, DNA synthesis can be measured by quantitative PCR at standard reaction conditions, as compared to the base construct RTase. Consistent with assessments of thermostability, quantitative comparisons are made under similar or the same reaction conditions and the amount of cDNA synthesized using the base construct RTase is compared to the amount of cDNA produced using the RTase mutant (*see* Tables 4, 5, 6, and 7). In some embodiments, the RTase mutant of the disclosure with enhanced DNA synthesis may produce about 5% to about 200% more cDNA than the base construct RTase. In some embodiments, the RTase mutant of the disclosure with enhanced DNA synthesis has at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or 200% more than, or at least 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold, or more than 10-fold more DNA synthesis than the RTase base construct DNA synthesis.

Reverse transcriptase activity, as described herein, was evaluated in a one-step or two-step procedure. The one-step procedure combines reverse transcription and quantitative PCR in a single reaction. The method is performed by including Gene Expression Master Mix, RTase, RNA, a fluorescent probe, and primers and probes as described in Example 3. The two-step procedure comprises reverse transcription followed by quantitative PCR. In the reverse transcription step, RTase is added to a mixture containing RNA, gene specific primers, first strand synthesis buffer, and RNase. The resultant cDNA is then quantified in a second step wherein the cDNA is combined with Gene Expression Master Mix, primers and probes, and a fluorescent marker. The cDNA produced in either the one-step and two-step procedures is quantified, and the mean and standard deviation reported as shown herein in Tables 4, 5, 6, and 7.

As used herein, "RNase H activity" refers to cleavage of RNA in DNA-RNA duplexes via a hydrolytic mechanism to produce 5' phosphate terminated oligonucleotides. RNase H activity does not include degradation of single-stranded nucleic acids, duplex DNA, or

double-stranded RNA. As used herein, the phrase "substantially lacks RNase H activity" means having less than 10%, 5%, 1%, 0.5%, or 0.1% of the activity of a wild type enzyme. As used herein, the phrase "lacks RNase H activity" means having undetectable RNase H activity or having less than about 1%, 0.5%, or 0.1% of the RNase H activity of a wild type enzyme.

As used herein, the term "mutation" refers to a change introduced into the nucleic acid sequence encoding a protein that changes the amino acid sequence of the protein, including but not limited to substitutions, insertions, deletions, point mutations, transpositions, inversions, frame shifts, nonsense mutations, truncations, or other forms of aberrations. A mutation may produce no discernible changes or result in a new property, function, or trait of the mutated protein. An RTase mutant of the disclosure may have one or more mutations in the nucleic acid sequence encoding the RTase mutant resulting in one or more mutations in the amino acid sequence of the RTase mutant. A mutation can result in one or more amino acids being substituted for an alternate amino acid residue, including Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and/or Val. The resulting amino acid mutations may impart altered functional and biological properties to the RTase mutant including but not limited to increased activity, enhanced DNA synthesis, enhanced stability or enhanced thermostability, reduced or eliminated RNase H activity, reduced terminal deoxynucleotidyl transferase activity, increased accuracy or increased fidelity, increased specificity, or altered half-life.

As used herein, the terms "detecting," "detection," "determining," and the like refer to assays performed for identification of the quantity of cDNA synthesis as a marker of RTase activity. The amount of marker expression or activity detected in the sample can be the same as, decreased, or increased as compared to the amount of marker expression or activity detected using the RTase base construct. One of skill in the art will understand that amount of cDNA can be quantified using multiple techniques.

The term "increased," as used herein with regard to RTase activity, refers to the level of RTase activity of an RTase mutant as compared to the RTase base construct. An RTase mutant has "increased" RTase activity if the level of its RTase activity, as measured by the quantity of cDNA synthesized or as measured by other methods known in the art, is more than the RTase base construct activity. For example, the RTase activity of the RTase mutant is increased if the RTase activity is at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% more than, or at least 2-

fold, 3-fold, 4-fold, 5-fold, or 10-fold, or more than 10-fold more than the RTase base construct activity.

The term "decreased," as used herein with regard to RTase activity, refers to the level of RTase activity of an RTase mutant as compared to the RTase base construct. An RTase mutant has "decreased" RTase activity if the level of its RTase activity, as measured by the quantity of cDNA synthesized or as measured by other methods known in the art is less than the RTase base construct activity. For example, the RTase activity of the RTase mutant is decreased if the RTase activity is at least 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% less than, or at least 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, or more than 10-fold less than the RTase base construct activity.

As used herein, the term "amplification" refers to any known *in vitro* procedure for obtaining multiple copies of a target nucleic acid sequence or its complement or fragments thereof. *In vitro* amplification refers to production of an amplified nucleic acid that may contain less than the complete target region sequence or its complement. Known *in vitro* amplification methods include, for example, transcription-mediated amplification, replicase-mediated amplification, polymerase chain reaction (PCR) amplification, ligase chain reaction (LCR) amplification, and strand-displacement amplification (SDA, including multiple strand-displacement amplification method (MSDA)). Replicase-mediated amplification uses self-replicating RNA molecules, and a replicase such as Q- β -replicase. PCR amplification uses DNA polymerase, primers, and thermal cycling to synthesize multiple copies of the two complementary strands of DNA or cDNA. PCR involves denaturation of a double-stranded DNA molecule, followed by annealing of DNA primers directed to the sequence of interest, and amplification/extension of the newly formed DNA strand. LCR amplification uses at least four separate oligonucleotides to amplify a target and its complementary strand by using multiple cycles of hybridization, ligation, and denaturation. SDA is a method in which a primer contains a recognition site for a restriction endonuclease that permits the endonuclease to nick one strand of a hemimodified DNA duplex that includes the target sequence, followed by amplification in a series of primer extension and strand displacement steps. Other strand-displacement amplification methods known in the art (*e.g.*, MSDA) do not require endonuclease nicking. Those of skill in the art will understand that the oligonucleotide primer sequences of the disclosure may be readily used in any *in vitro* amplification method based on primer extension by a polymerase. As commonly known in the art, oligonucleotides are designed to bind to a complementary sequence under selected conditions.

As used herein, "real time PCR" or "quantitative PCR" refers to a PCR method wherein the amount of product being formed can be monitored using fluorescent probes and quantified by tracking the fluorescent signal produced, above a threshold level. Real time PCR can be performed in a one-step reaction that includes the reverse transcription step in a simultaneous reaction (*i.e.*, real time PCR or RT-PCR) or in a two-step reaction in which the reverse transcription step and PCR steps are performed consecutively.

As used herein, the term "complementary" refers to the broad concept of sequence complementarity between regions of two nucleic acid strands or between two regions of the same nucleic acid strand. A first region of a nucleic acid is complementary to a second region of the same or a different nucleic acid if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide of the first region is capable of base pairing with a nucleotide of the second region. In some embodiments, the first region comprises a first portion and the second region comprises a second portion, whereby, when the first and second portions are arranged in an antiparallel fashion, at least about 50%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the nucleotides of the first portion are capable of base pairing with nucleotides in the second portion. In another embodiment, all nucleotides of the first portion are capable of base pairing with nucleotides in the second portion.

Polypeptide and polynucleotide sequences may be aligned, and percentages of identical amino acids or nucleotides in a specified region may be determined against another polypeptide or polynucleotide sequence, using computer algorithms that are publicly available. The percent identity of a polynucleotide or polypeptide sequence is determined by aligning polynucleotide and polypeptide sequences using appropriate algorithms, such as BLASTN or BLASTP, respectively, set to default parameters; identifying the number of identical nucleic or amino acids over the aligned portions; dividing the number of identical nucleic or amino acids by the total number of nucleic or amino acids of the polynucleotide or polypeptide of the disclosure; and then multiplying by 100 to determine the percent identity.

As used herein, the terms "sample" and "biological sample" include a specimen or culture obtained from any source. Biological samples can be obtained from cerebrospinal fluid, lacrimal fluid, blood (including any blood product, such as whole blood, plasma, serum, or specific types of cells of the blood), urine, saliva, and the like. Biological samples also include tissue samples, such as biopsy tissues or pathological tissues that have previously been fixed (*e.g.*, formaline snap frozen, cytological processing).

2. Reverse Transcriptases

The disclosure relates to Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutants. The MMLV RTase mutants of the disclosure are prepared by modifying the sequence of an MMLV RTase base construct (SEQ ID NO: 637). In one embodiment, the MMLV RTase mutant of the disclosure comprises the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least one amino acid substitution that is: (a) an isoleucine to arginine, lysine or methionine substitution at position 61 (I61R, I61K or I61M); (b) a glutamine to arginine, lysine or isoleucine substitution at position 68 (Q68R, Q68K or Q68I); (c) a glutamine to arginine, histidine or isoleucine substitution at position 79 (Q79R, Q79H or Q79I); (d) a leucine to arginine, lysine or asparagine substitution at position 99 (L99R, L99K or L99N); (e) a glutamic acid to aspartic acid, methionine or typtophan substitution at position 282 (E282D, E282M or E282W); and/or (f) an arginine to alanine substitution at position 298 (R298A).

In another embodiment, the MMLV RTase mutant of the disclosure comprises the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least two amino acid substitutions that are: (a) an isoleucine to arginine substitution at position 61 (I61R); (b) a glutamine to arginine substitution at position 68 (Q68R); (c) a glutamine to arginine substitution at position 79 (Q79R); (d) a leucine to arginine substitution at position 99 (L99R); (e) a glutamic acid to aspartic acid substitution at position 282 (E282D); and/or (f) an arginine to alanine substitution at position 298 (R298A): (a) an isoleucine to arginine substitution at position 61 and a glutamic acid to aspartic acid substitution at position 282 (I61R/E282D); (b) a leucine to arginine at substitution position 99 and a glutamic acid to aspartic acid substitution at position 282 (L99R/E282D); (c) a glutamine to arginine substitution at position 68 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/E282D); (d) a glutamine to arginine substitution at position 79 and a glutamic acid to aspartic acid substitution at position 282 (Q79R/E282D); (e) a glutamic acid to aspartic acid substitution at position 282 and an arginine to alanine substitution at position 298 (E282D/R298A); (f) an isoleucine to arginine substitution at position 61 and a leucine to arginine substitution at position 99 (I61R/L99R); (g) an isoleucine to arginine substitution at position 61 and a glutamine to arginine substitution at position 68 (I61R/Q68R); (h) an isoleucine to arginine substitution at position 61 and a glutamine to arginine substitution at position 79 (I61R/Q79R); (i) an isoleucine to arginine substitution at position 61 and an arginine to alanine substitution at position 298 (I61R/R298A); (j) a glutamine to arginine substitution at position 68 and a leucine to arginine

substitution at position 99 (Q68R/L99R); (k) a glutamine to arginine substitution at position 79 and a leucine to arginine substitution at position 99 (Q79R/L99R); (l) a leucine to arginine at substitution position 99 and an arginine to alanine substitution at position 298 (L99R/R298A); (m) a glutamine to arginine substitution at position 68 and a glutamine to arginine substitution at position 79 (Q68R/Q79R); (n) a glutamine to arginine substitution at position 68 and an arginine to alanine substitution at position 298 (Q68R/R298A); or (o) a glutamine to arginine substitution at position 79 and an arginine to alanine substitution at position 298 (Q79R/R298A).

In another embodiment, the MMLV RTase mutant of the disclosure comprises the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least three amino acid substitutions that are: (a) a glutamine to arginine substitution at position 68 (Q68R); (b) a glutamine to arginine substitution at position 79 (Q79R); (c) a leucine to arginine substitution at position 99 (L99R); and/or (d) a glutamic acid to aspartic acid substitution at position 282 (E282D): (a) a glutamine to arginine substitution at position 68, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/L99R/E282D); (b) a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q79R/L99R/E282D); (c) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 68 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79R/E282D); or (d) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 68 and a leucine to arginine substitution at position 99 (Q68R/Q79R/L99R).

In another embodiment, the MMLV RTase mutant of the disclosure comprises the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least four amino acid substitutions that are: (a) a glutamine to arginine, lysine or isoleucine substitution at position 68 (Q68R, Q68K or Q68I); (b) a glutamine to arginine, histidine or isoleucine substitution at position 79 (Q79R, Q79H or Q79I); (c) a leucine to arginine, lysine or asparagine substitution at position 99 (L99R, L99K or L99N); (d) a glutamic acid to aspartic acid, methionine or typtophan substitution at position 282 (E282D, E282M or E282W): (a) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79R/L99R/E282D); (b) a glutamine to arginine substitution at position 68, a

glutamine to arginine substitution at position 79, a leucine to lysine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79R/L99K/E282D); (c) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to asparagine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79R/L99N/E282D); (d) a glutamine to isoleucine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68I/Q79R/L99R/E282D); (e) a glutamine to lysine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68K/Q79R/L99R/E282D); (f) a glutamine to arginine substitution at position 68, a glutamine to histidine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79H/L99R/E282D); (g) a glutamine to arginine substitution at position 68, a glutamine to isoleucine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/Q79I/L99R/E282D); (h) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to methionine substitution at position 282 (Q68R/Q79R/L99R/E282M); (i) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to tryptophan substitution at position 282 (Q68R/Q79R/L99R/E282W); or (j) a glutamine to isoleucine substitution at position 68, a glutamine to histidine substitution at position 79, a leucine to lysine substitution at position 99 and a glutamic acid to methionine substitution at position 282 (Q68I/Q79H/L99K/E282M).

In another embodiment, the MMLV RTase mutant of the disclosure comprises the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least five amino acid substitutions that are: (a) an isoleucine to lysine or methionine substitution at position 61 (I61K or I61M); (b) a glutamine to arginine or isoleucine substitution at position 68 (Q68R or Q68I); (c) a glutamine to arginine or histidine substitution at position 79 (Q79R or Q79H); (d) a leucine to arginine or lysine substitution at position 99 (L99R or L99K); (e) a glutamic acid to aspartic acid or methionine substitution at position 282 (E282D or E282M): (a) an isoleucine to lysine

substitution at position 61, a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (I61K/Q68R/Q79R/L99R/E282D); (b) an isoleucine to methionine substitution at position 61, a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282 (I61M/Q68R/Q79R/L99R/E282D); or (c) an isoleucine to methionine substitution at position 61, a glutamine to isoleucine substitution at position 68, a glutamine to histidine substitution at position 79, a leucine to lysine substitution at position 99 and a glutamic acid to methionine substitution at position 282 (I61M/Q68IR/Q79H/L99K/E282M).

In another embodiment, the MMLV RTase mutant of the disclosure comprises the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least five or more amino acid substitutions that are : (a) a glutamine to arginine, lysine or isoleucine substitution at position 68 (Q68R, Q68K or Q68I); (b) a glutamine to arginine, histidine or isoleucine substitution at position 79 (Q79R, Q79H or Q79I); (c) a leucine to arginine, lysine or asparagine substitution at position 99 (L99R, L99K or L99N); (d) a glutamic acid to aspartic acid, methionine or typtophan substitution at position 282 (E282D, E282M or E282W); (e) a glutamine to glutamic acid substitution at position 299; (f) threonine to glutamic acid substitution at position 332; (g) valine to arginine substitution at position 433; (h) isoleucine to glutamic acid substitution at position 593; (a) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282, a glutamine to glutamic acid substitution at position 299, a valine to arginine substitution at position 433 and a isoleucine to glutamic acid at position 593 (Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E); (b) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to argine substitution at postion 82, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282, a glutamine to glutamic acid substitution at position 299, a valine to arginine substitution at position 433 and a isoleucine to glutamic acid at position 593 (Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E); (c) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to argine substitution at postion 82, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282, a glutamine to glutamic acid substitution at position 299, a threonine to glutamic acid substitution at position 332, and a

isoleucine to glutamic acid at position 593

(Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E); (d) a glutamine to arginine substitution at position 68, a glutamine to arginine substitution at position 79, a leucine to arginine substitution at position 82, a leucine to arginine substitution at position 99 and a glutamic acid to aspartic acid substitution at position 282, a glutamine to glutamic acid substitution at position 299, a threonine to glutamic acid substitution at position 332, a valine to arginine substitution at position 433, and a isoleucine to glutamic acid at position 593 (Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E)

In one embodiment the RTase mutant amino acid sequence comprises a mutant selected from Table 3, Table 8, Table 9, Table 12, or Table 33. In one aspect the RTase mutant amino acid sequence comprises a mutant selected from SEQ ID NO: 638, SEQ ID NO: 639, SEQ ID NO: 640, SEQ ID NO: 641, SEQ ID NO: 642, SEQ ID NO: 643, SEQ ID NO: 644, SEQ ID NO: 645, SEQ ID NO: 646, SEQ ID NO: 647, SEQ ID NO: 648, SEQ ID NO: 649, SEQ ID NO: 650, SEQ ID NO: 651, SEQ ID NO: 652, SEQ ID NO: 653, SEQ ID NO: 654, SEQ ID NO: 655, SEQ ID NO: 656, SEQ ID NO: 657, SEQ ID NO: 658, SEQ ID NO: 659, SEQ ID NO: 660, SEQ ID NO: 661, SEQ ID NO: 662, SEQ ID NO: 663, SEQ ID NO: 664, SEQ ID NO: 665, SEQ ID NO: 666, SEQ ID NO: 667, SEQ ID NO: 668, SEQ ID NO: 669, SEQ ID NO: 670, SEQ ID NO: 671, SEQ ID NO: 672, SEQ ID NO: 673, SEQ ID NO: 674, SEQ ID NO: 675, SEQ ID NO: 676, SEQ ID NO: 677, SEQ ID NO: 678, SEQ ID NO: 679, SEQ ID NO: 680, SEQ ID NO: 681, SEQ ID NO: 682, SEQ ID NO: 683, SEQ ID NO: 684, SEQ ID NO: 685, SEQ ID NO: 686, SEQ ID NO: 687, SEQ ID NO: 688, SEQ ID NO: 689, SEQ ID NO: 690, SEQ ID NO: 691, SEQ ID NO: 692, SEQ ID NO: 693, SEQ ID NO: 694, SEQ ID NO: 695, SEQ ID NO: 696, SEQ ID NO: 697, SEQ ID NO: 698, or SEQ ID NO: 699.

In one embodiment the RTase mutant amino acid sequence comprises a C-terminal extension. In one aspect the C-terminal extension comprises a peptide sequence. In another embodiment an isolated polypeptide encodes a RTase mutant with a C-terminal extension

The claimed invention is based, at least in part, on the discovery that certain single and double amino acid mutations introduced into an MMLV RTase sequence, as disclosed herein, result in an MMLV RTase with increased or enhanced thermostability and/or RTase activity. Accordingly, methods for synthesizing the MMLV RTase mutants and methods for performing reverse transcription-polymerase chain reaction (RT-PCR) are also provided

herein. Further provided are kits comprising the isolated MMLV RTase single, double, triple, or more mutations.

In certain embodiments, the mutated RTase is derived from the retrovirus Moloney murine leukemia virus (MMLV). In other embodiments, a mutated RTase of the disclosure could be derived from the RTase from a retrovirus other than MMLV, such as avian myeloblastosis virus (AMV) or human immunodeficiency virus type 1 (HIV-1), by introducing the same mutations into an RTase base construct obtained from the other retrovirus.

In certain embodiments, the RTase mutants of the disclosure are obtained by genetic engineering techniques that are well known in the art. For example, site-directed and random mutagenesis can be used to generate the RTase mutants of the disclosure.

In one embodiment of the disclosure, an RTase mutant of the disclosure is part of a composition.

3. Mutagenesis

The RTase mutants of the disclosure can be prepared by standard methods disclosed herein or known in the art. In one embodiment, the nucleic acid sequence of the RTase base construct (SEQ ID NO: 637) is modified to create a nucleic acid sequence encoding an RTase mutant. One of skill in the art will recognize that colonies with the appropriate strains can be used to grow and express an RTase mutant of interest, and following cell harvest and protein isolation, the RTase mutant can be used in cDNA synthesis techniques. Non-limiting examples of mutagenesis and cDNA synthesis are described herein in Examples 1-3.

As used herein, the term "mutagenesis" refers to the introduction of a genetic change in the nucleic acid sequence of a cell, wherein the alteration is then inherited by each cell. One of skill in the art will understand that mutations in a given nucleic acid sequence can be introduced using a variety of methods. One of skill in the art will further recognize that mutagenesis methods seek to mutate a target gene or target polynucleotide. The target gene may encode any one or more desired proteins. Mutagenesis methods commonly use a synthetic oligonucleotide that carries the desired sequence modification. The mutagenic oligonucleotide is incorporated into the DNA sequence using *in vitro* enzymatic DNA synthesis and is propagated in a mutant or wild-type bacterium.

Site directed mutagenesis, wherein targeted mutations are introduced into one or more desired positions of a template polynucleotide, may be achieved using primer extension mutagenesis. This technique requires the use of a specific primer that contains one or more

desired mutations relative to the template polynucleotide. The mutagenesis primer can be a synthetic oligonucleotide or a PCR product. The mutated primer may include one or more substitutions, deletions, additions, or combinations thereof.

Mutated reverse transcriptases may also be generated using random mutagenesis, wherein mutations are introduced into the mutagenesis primer during synthesis. Randomly mutagenized oligonucleotides may also be used as mutagenesis primers.

In another embodiment, the mutated reverse transcriptases of the disclosure can be developed using error-prone rolling circle amplification (RCA). In this technique, the fidelity of a DNA polymerase is decreased by performing the RCA in the presence of $MnCl_2$ or by decreasing the amount of input DNA.

4. cDNA Synthesis

The disclosure also relates to the activity of MMLV RTases, as measured by the quantity of cDNA produced by the MMLV RTases disclosed herein. cDNA can be prepared using one-step or two-step procedures and can be obtained from a variety of template molecules. As used herein, the term "template molecule" refers to a biological molecule that carries the genetic code for use in making a new nucleic acid strand. For example, in DNA replication, the unwound double helix and each single-stranded DNA molecule is used as a template to synthesize a complementary strand. Reverse transcription generates cDNA from RNA. One of skill in the art will understand that cDNA molecules may be prepared from a variety of nucleic acid template molecules. In one embodiment, the nucleic acid template can be single-stranded or double-stranded DNA. In one embodiment, RNA can be used in cDNA synthesis. In certain embodiments, the MMLV RTase mutants of the disclosure exhibit increased or enhanced thermostability and/or RTase activity as compared to an RTase base construct. In other embodiments, the MMLV RTase mutants of the disclosure exhibit altered half-life, reduced or eliminated RNase H activity, reduced terminal deoxynucleotidyl transferase activity, increased accuracy or fidelity, or increased specificity.

The disclosure also provides methods for synthesizing cDNA using the MMLV RTase mutants of the disclosure that have single or double amino acid mutations. The MMLV RTase mutants of the disclosure may be used in methods that produce a first strand cDNA or a first and second strand cDNA. One of skill in the art will understand that first and second strand cDNA may form a double-stranded DNA molecule, which may include a full-length cDNA sequence and cDNA libraries.

The cDNA molecules that have been reverse transcribed by the MMLV RTase mutants of the disclosure may be isolated, or the reaction mixture containing the cDNA molecules may be directly used in downstream applications or for further analysis or manipulation. Amplification methods that may be used to practice the methods of the disclosure are described herein and are well known in the art. Reverse transcription reactions may be carried out using non-specific primers, such as an anchored oligo-dT primer, or random sequence primers, or using a target-specific primer complementary to the RNA for each genetic probe being monitored, or using thermostable DNA polymerases (such as AMV RTase or MMLV RTase).

Amplification methods utilize pairs of primers that selectively hybridize to nucleic acids corresponding to a specific nucleotide sequence of interest that are contacted with the isolated nucleic acid under conditions that permit selective hybridization. Once hybridized, the nucleic acid:primer complex is contacted with one or more enzymes that facilitate template-dependent nucleic acid synthesis. Multiple rounds of amplification, also referred to as "cycles," are conducted until a sufficient amount of amplification product is produced. Next, the amplification product is detected. In certain methods, the detection may be performed by visual means. Alternatively, the detection may involve indirect identification of the product via chemiluminescence, radioactive scintigraphy of incorporated radiolabel or fluorescent label, or even via a system using electrical or thermal impulse signals.

Methods based on ligation of two (or more) oligonucleotides in the presence of a nucleic acid having the sequence of the resulting "di-oligonucleotide," thereby amplifying the di-oligonucleotide, also may be used in the amplification step of the disclosure.

In some embodiments of the disclosure, the detection process can utilize a hybridization technique, for example, wherein a specific primer or probe is selected to anneal to a target biomarker of interest, and thereafter detection of selective hybridization is made. As commonly known in the art, the oligonucleotide probes and primers can be designed by taking into consideration the melting point of hybridization thereof with its targeted sequence.

One of skill in the art will recognize that cDNA molecules made using the MMLV RTase mutants of the disclosure can be used in a variety of additional downstream applications. For example, amplification methods may include one-step PCR, two-step PCR, real-time or quantitative PCR, hot-start PCR, nested PCR, touch down PCR, differential display PCR (DDRT-PCR), microarray technologies, inverse PCR, Rapid amplification of PCR ends (RACE or anchored PCR), multiplex PCR, and site directed PCR mutagenesis.

Synthesized cDNA and cDNA libraries created with the MMLV RTase mutants of the disclosure can be used in cloning and/or sequencing for further characterization. One of skill in the art will recognize that nucleic acid amplification using cDNA prepared with the MMLV RTase mutants of the disclosure may include additional techniques not listed herein.

To enable hybridization to occur under the methods presented above, oligonucleotide primers and probes should comprise an oligonucleotide sequence that has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a portion of the sequence of interest.

5. Biological Samples

The MMLV RTase mutants and associated methods of the disclosure may be practiced with any suitable biological sample from which RNA or DNA can be isolated. In one embodiment of the disclosure, the biological sample may be a bodily fluid or tissue obtained from either a diseased or a healthy subject. In some embodiments of the disclosure, the biological sample may be a bodily fluid, including but not limited to whole blood, plasma, serum, feces, or urine. In another embodiment, the methods of the disclosure may be practiced with any suitable samples that are freshly isolated or that have been frozen or stored after having been collected from a subject, for example, with a known diagnosis, treatment, and/or outcome history. Samples may be collected by any non-invasive means, such as, for example, fine needle aspiration or needle biopsy, or alternatively, by an invasive method, including, for example, surgical biopsy. In such embodiments, RNA or DNA can be extracted from a biological sample (*e.g.*, blood serum) before analysis. Methods of RNA and DNA extraction are well known in the art.

A number of kits for use in extracting RNA (*i.e.*, total RNA or mRNA) from bodily fluids or tissues (*e.g.*, blood serum) and are known in the art and commercially available. One of ordinary skill in the art can easily select an appropriate kit for a particular situation.

In certain embodiments of the disclosure, after extraction, mRNA is amplified, and transcribed into cDNA, which can then serve as template for multiple rounds of transcription by the appropriate RNA polymerase. Amplification methods that may be used to practice the methods of the disclosure are described herein and are well known in the art. Reverse transcription reactions may be carried out using non-specific primers, such as an anchored oligo-dT primer, or random sequence primers, or using a target-specific primer complementary to the RNA for each genetic probe being monitored, or using thermostable DNA polymerases, such as MMLV RTase or the MMLV RTase mutants of the disclosure.

In certain embodiments, the RNA isolated from a biological sample (*e.g.*, after amplification and/or conversion to cDNA or cRNA) is labeled with a detectable agent before being analyzed. The role of a detectable agent is to facilitate detection of RNA or to allow visualization of hybridized nucleic acid fragments (*e.g.*, nucleic acid fragments hybridized to genetic probes in an array-based assay). In some embodiments, the detectable agent is selected such that it generates a signal which can be measured and whose intensity is related to the amount of labeled nucleic acids present in the sample being analyzed.

Methods for labeling nucleic acid molecules are well known in the art. A review of labeling protocols and label detection techniques can be found in Kricka, *Ann. Clin. Biochem.* 39: 114-29 (2002); van Gijlswijk *et al.*, *Expert Rev. Mol. Diagn.* 1: 81-91 (2001); and Joos *et al.*, *J. Biotechnol.* 35: 135-53 (1994). Standard nucleic acid labeling methods include incorporation of radioactive agents; direct attachment of fluorescent dyes or of enzymes; chemical modifications of nucleic acid fragments making them detectable immunochemically or by other affinity reactions; and enzyme-mediated labeling methods, such as random priming, nick translation, PCR, and tailing with terminal transferase.

Any of a wide variety of detectable agents can be used to practice the methods of the disclosure. Suitable detectable agents include but are not limited to various ligands, radionuclides, fluorescent dyes, chemiluminescent agents, microparticles (such as, for example, quantum dots, nanocrystals, and phosphors), enzymes (such as, for example, those used in an ELISA, *i.e.*, horseradish peroxidase, beta-galactosidase, luciferase, and alkaline phosphatase), colorimetric labels, magnetic labels, biotin, dioxigenin, or other haptens and proteins for which antisera or monoclonal antibodies are available.

6. Kits

The disclosure also provides kits for use in reverse transcription or related technologies. These kits include one or more of the following: an MMLV RTase mutant enzyme, reagents and buffers for conducting a reverse transcriptase reaction, a box, vial tubes, ampules, and the like. Kits can also include instructions for use of the kit for practicing any of the methods disclosed herein or other methods known to those of skill in the art.

EXAMPLES

The claimed invention is further illustrated by the following Examples, which should not be construed as limiting. Those of skill in the art will recognize that the claimed

invention may be practiced with variations of the disclosed structures, materials, compositions, and methods, and such variations are regarded as within the scope of the claimed invention.

The RTases described herein were overexpressed in *E. coli*, purified to homogeneity, and tested for their ability to enhance RNA detection in the context of reverse transcriptase quantitative PCR (RT-qPCR).

Example 1. Preparation of Reverse Transcriptase Mutants by Site Directed Mutagenesis

a. Cloning of MMLV RTase mutants created from base construct (RNase H minus construct)

MMLV RTase mutants were prepared by first introducing three mutations (D524G, E562Q, and D583N) into the amino acid sequence of the wild-type, or naturally occurring, MMLV RTase to prepare an MMLV RTase base construct (SEQ ID NO: 637). The three mutations, which are contained in the SuperScript II RTase (Invitrogen), have been shown to reduce RNase H activity (*see* U.S. Patent No. 5,405,776). The MMLV RTase base construct was optimized for *E. coli* expression and obtained as gBlocks® Gene Fragments (Integrated DNA Technologies) or by custom gene synthesis with the appropriate purification tag. Subsequent genes were amplified using standard PCR conditions and primers (*see* Table 1). Amplified DNA was subjected to purification using a QIAquick PCR Purification kit (Qiagen, Catalog #28104), followed by gene fragment assembly into a pET28b expression plasmid. Plasmid DNA was isolated and sequenced to verify the desired sequence following transformation into *E. coli* cells. MMLV RTase mutations were selected by rational design (Figures 1A-1C) and introduced by site-directed mutagenesis, using standard PCR conditions and primers (*see* Table 1). Resulting plasmids were transformed into *E. coli* BL21(DE3) cells for expression.

Table 1. Sequences of primers used for cloning of MMLV RTase base constructs and mutants into pET28b.

SEQ ID NO:	Primer Name	Primer Sequence (5'–3')
1	pET28b 5' Reverse	GGTATATCTCCTTCTTAAAGTTAAACAAAATTATT TCTAGAGGGGAAT
2	pET28b 3' Forward	GATCCGGCTGCTAACAAAGCC
3	MMLV 5' Primer	TTTTGTTTAACTTTAAGAAGGAGATATACCATGGG CAGCAGCCATCATCATC
4	MMLV 3' Primer	GCAGCCAACTCAGCTTCCTTTCGGGCTTTGTTAAA AATGCTCGCTAGTGTAGGGAGAGC
5	MMLV K53A Top SDM	AAGCACCGTTGATCATCCCGTTAGCGGCAACGTCT ACACCTGTCTCTATCAAAC
6	MMLV K53R Top SDM	AAGCACCGTTGATCATCCCGTTACGTGCAACGTCT ACACCTGTCTCTATCAAAC
7	MMLV K53E Top SDM	AAGCACCGTTGATCATCCCGTTAGAAGCAACGTCT ACACCTGTCTCTATCAAAC

8	MMLV T55A Top SDM	CCGTTGATCATCCCGTTAAAGGCAGCGTCTACACC TGTCTCTATCAAACAGTACCCC
9	MMLV T55R Top SDM	CCGTTGATCATCCCGTTAAAGGCAGGTTCTACACC TGTCTCTATCAAACAGTACCCC
10	MMLV T55E Top SDM	CCGTTGATCATCCCGTTAAAGGCAGAATCTACACC TGTCTCTATCAAACAGTACCCC
11	MMLV T57A Top SDM	ATCATCCCGTTAAAGGCAACGTCTGCGCCTGTCTC TATCAAACAGTACCCCATGAG
12	MMLV T57R Top SDM	ATCATCCCGTTAAAGGCAACGTCTCGTCCTGTCTC TATCAAACAGTACCCCATGAG
13	MMLV T57E Top SDM	ATCATCCCGTTAAAGGCAACGTCTGAACCTGTCTC TATCAAACAGTACCCCATGAG
14	MMLV V59A Top SDM	CCGTTAAAGGCAACGTCTACACCTGCGTCTATCAA ACAGTACCCCATGAGTCAAGAGG
15	MMLV V59R Top SDM	CCGTTAAAGGCAACGTCTACACCTCGTTCTATCAA ACAGTACCCCATGAGTCAAGAGG
16	MMLV V59E Top SDM	CCGTTAAAGGCAACGTCTACACCTGAATCTATCAA ACAGTACCCCATGAGTCAAGAGG
17	MMLV I61A Top SDM	TAAAGGCAACGTCTACACCTGTCTCTGCGAAACAG TACCCCATGAGTCAAGAGG
18	MMLV I61R Top SDM	TAAAGGCAACGTCTACACCTGTCTCTCGTAAACAG TACCCCATGAGTCAAGAGG
19	MMLV I61E Top SDM	TAAAGGCAACGTCTACACCTGTCTCTGAAAAACAG TACCCCATGAGTCAAGAGG
20	MMLV K62A Top SDM	GGCAACGTCTACACCTGTCTCTATCGCGCAGTACC CCATGAGTCAAGAGGC
21	MMLV K62R Top SDM	GGCAACGTCTACACCTGTCTCTATCCGTCAGTACC CCATGAGTCAAGAGGC
22	MMLV K62E Top SDM	GGCAACGTCTACACCTGTCTCTATCGAACAGTACC CCATGAGTCAAGAGGC
23	MMLV Q68A Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTGCGGAG GCCCCGCTGGG
24	MMLV Q68R Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTCGTGAG GCCCCGCTGGG
25	MMLV Q68E Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTGAAGAG GCCCCGCTGGG
26	MMLV K75A Top SDM	GGCCCGCCTGGGGATTGCGCCACATATTCAGCGCT TGCTGGACCA
27	MMLV K75R Top SDM	GGCCCGCCTGGGGATTGCTCCACATATTCAGCGCT TGCTGGACCA
28	MMLV K75E Top SDM	GGCCCGCCTGGGGATTGAACCACATATTCAGCGCT TGCTGGACCA
29	MMLV Q79A Top	CGCCTGGGGATTAAAGCCACATATTGCGCGCTTGCT

	SDM	GGACCAGGGG
30	MMLV Q79R Top SDM	CGCCTGGGGATTAAGCCACATATTCGTGCTTGCT GGACCAGGGG
31	MMLV Q79E Top SDM	CGCCTGGGGATTAAGCCACATATTGAACGCTTGCT GGACCAGGGG
32	MMLV L99A Top SDM	CCGTGGAACACCCCCCTTGCGCCCGTGAAAAAGCC AGGTACAAAC
33	MMLV L99R Top SDM	CCGTGGAACACCCCCCTTCGTCCCGTGAAAAAGCC AGGTACAAAC
34	MMLV L99E Top SDM	CCGTGGAACACCCCCCTTGAACCCGTGAAAAAGCC AGGTACAAAC
35	MMLV V101A Top SDM	CACCCCCCTTCTGCCCCGCGAAAAAGCCAGGTACAA ACGATTATCGTCC
36	MMLV V101R Top SDM	CACCCCCCTTCTGCCCCGTAAAAAGCCAGGTACAA ACGATTATCGTCC
37	MMLV V101E Top SDM	CACCCCCCTTCTGCCCCGAAAAAAGCCAGGTACAA ACGATTATCGTCC
38	MMLV K102A Top SDM	CCCCCTTCTGCCCCGTGGCGAAGCCAGGTACAAACG ATTATCGTCC
39	MMLV K102R Top SDM	CCCCCTTCTGCCCCGTGCGTAAGCCAGGTACAAACG ATTATCGTCC
40	MMLV K102E Top SDM	CCCCCTTCTGCCCCGTGGAAAAGCCAGGTACAAACG ATTATCGTCC
41	MMLV K103A Top SDM	CCCCCTTCTGCCCCGTGAAAGCGCCAGGTACAAACG ATTATCGTCCAGTT
42	MMLV K103R Top SDM	CCCCCTTCTGCCCCGTGAAACGTCCAGGTACAAACG ATTATCGTCCAGTT
43	MMLV K103E Top SDM	CCCCCTTCTGCCCCGTGAAAGAACCAGGTACAAACG ATTATCGTCCAGTT
44	MMLV T106A Top SDM	GCCCGTGAAAAAGCCAGGTGCGAACGATTATCGTC CAGTTCAAGATCTTCG
45	MMLV T106R Top SDM	GCCCGTGAAAAAGCCAGGTGCGTAACGATTATCGTC CAGTTCAAGATCTTCG
46	MMLV T106E Top SDM	GCCCGTGAAAAAGCCAGGTGAAAACGATTATCGTC CAGTTCAAGATCTTCG
47	MMLV N107A Top SDM	CCCGTGAAAAAGCCAGGTACAGCGGATTATCGTCC AGTTCAAGATCTTCGCG
48	MMLV N107R Top SDM	CCCGTGAAAAAGCCAGGTACACGTGATTATCGTCC AGTTCAAGATCTTCGCG
49	MMLV N107E Top SDM	CCCGTGAAAAAGCCAGGTACAGAAGATTATCGTCC AGTTCAAGATCTTCGCG
50	MMLV Y109A Top SDM	CGTGAAAAAGCCAGGTACAAACGATGCGCGTCCAG TTCAAGATCTTCGCG

51	MMLV Y109R Top SDM	CGTGAAAAAGCCAGGTACAAACGATCGTCGTCCAG TTCAAGATCTTCGCG
52	MMLV Y109E Top SDM	CGTGAAAAAGCCAGGTACAAACGATGAACGTCCAG TTCAAGATCTTCGCG
53	MMLV R110A Top SDM	CGTGAAAAAGCCAGGTACAAACGATTATGCGCCAG TTCAAGATCTTCGCGAGG
54	MMLV R110K Top SDM	CGTGAAAAAGCCAGGTACAAACGATTATAAACCAG TTCAAGATCTTCGCGAGG
55	MMLV R110E Top SDM	CGTGAAAAAGCCAGGTACAAACGATTATGAACCAG TTCAAGATCTTCGCGAGG
56	MMLV V112A Top SDM	GCCAGGTACAAACGATTATCGTCCAGCGCAAGATC TTCGCGAGGTCAACAAAC
57	MMLV V112R Top SDM	GCCAGGTACAAACGATTATCGTCCACGTCAAGATC TTCGCGAGGTCAACAAAC
58	MMLV V112E Top SDM	GCCAGGTACAAACGATTATCGTCCAGAACAAGATC TTCGCGAGGTCAACAAAC
59	MMLV K120A Top SDM	AGTTCAAGATCTTCGCGAGGTCAACGCGCGCGTAG AAGACATCCATCCGAC
60	MMLV K120R Top SDM	AGTTCAAGATCTTCGCGAGGTCAACCGTCGCGTAG AAGACATCCATCCGAC
61	MMLV K120E Top SDM	AGTTCAAGATCTTCGCGAGGTCAACGAACGCGTAG AAGACATCCATCCGAC
62	MMLV E123A Top SDM	GCGAGGTCAACAAACGCGTAGCGGACATCCATCCG ACTGTACCTAATCC
63	MMLV E123R Top SDM	GCGAGGTCAACAAACGCGTAGCTGACATCCATCCG ACTGTACCTAATCC
64	MMLV E123D Top SDM	GCGAGGTCAACAAACGCGTAGATGACATCCATCCG ACTGTACCTAATCC
65	MMLV T128V Top SDM	ACGCGTAGAAGACATCCATCCGGTGGTACCTAATC CTTATAATCTGTTATCAGGCCTGC
66	MMLV T128R Top SDM	ACGCGTAGAAGACATCCATCCGCGGTGACCTAATC CTTATAATCTGTTATCAGGCCTGC
67	MMLV T128E Top SDM	ACGCGTAGAAGACATCCATCCGGAAGTACCTAATC CTTATAATCTGTTATCAGGCCTGC
68	MMLV K193A Top SDM	CGTCTGCCCCAGGGCTTTGCGAACAGCCCCACATT GTTCGATGAA
69	MMLV K193R Top SDM	CGTCTGCCCCAGGGCTTTGTAACAGCCCCACATT GTTCGATGAA
70	MMLV K193E Top SDM	CGTCTGCCCCAGGGCTTTGAAAACAGCCCCACATT GTTCGATGAA
71	MMLV E282A Top SDM	AGAAGGTCAACGTTGGCTGACTGCGGCGCGTAAGG AGACCGTAATG
72	MMLV E282R Top	AGAAGGTCAACGTTGGCTGACTCGTGCGCGTAAGG

	SDM	AGACCGTAATG
73	MMLV E282D Top SDM	AGAAGGTCAACGTTGGCTGACTGATGCGCGTAAGG AGACCGTAATG
74	MMLV A283V Top SDM	GAAGGTCAACGTTGGCTGACTGAAGTGCGTAAGGA GACCGTAATGGGGC
75	MMLV A283R Top SDM	GAAGGTCAACGTTGGCTGACTGAACGTCGTAAGGA GACCGTAATGGGGC
76	MMLV A283E Top SDM	GAAGGTCAACGTTGGCTGACTGAAGAACGTAAGGA GACCGTAATGGGGC
77	MMLV Q291A Top SDM	GCGTAAGGAGACCGTAATGGGGGCGCCTACGCCTA AGACGCCACG
78	MMLV Q291R Top SDM	GCGTAAGGAGACCGTAATGGGGCGTCCTACGCCTA AGACGCCACG
79	MMLV Q291E Top SDM	GCGTAAGGAGACCGTAATGGGGGAACCTACGCCTA AGACGCCACG
80	MMLV T293A Top SDM	GAGACCGTAATGGGGCAGCCTGCGCCTAAGACGCC ACGCCAGTTG
81	MMLV T293R Top SDM	GAGACCGTAATGGGGCAGCCTCGTCCTAAGACGCC ACGCCAGTTG
82	MMLV T293E Top SDM	GAGACCGTAATGGGGCAGCCTGAACCTAAGACGCC ACGCCAGTTG
83	MMLV K295A Top SDM	GTAATGGGGCAGCCTACGCCTGCGACGCCACGCCA GTTGCGTGAA
84	MMLV K295R Top SDM	GTAATGGGGCAGCCTACGCCTCGTACGCCACGCCA GTTGCGTGAA
85	MMLV K295E Top SDM	GTAATGGGGCAGCCTACGCCTGAAACGCCACGCCA GTTGCGTGAA
86	MMLV T296A Top SDM	TGGGGCAGCCTACGCCTAAGGCGCCACGCCAGTTG CGTGAATTTT
87	MMLV T296R Top SDM	TGGGGCAGCCTACGCCTAAGCGTCCACGCCAGTTG CGTGAATTTT
88	MMLV T296E Top SDM	TGGGGCAGCCTACGCCTAAGGAACCACGCCAGTTG CGTGAATTTT
89	MMLV R298A Top SDM	GCCTACGCCTAAGACGCCAGCGCAGTTGCGTGAAT TTTTGGGCACAG
90	MMLV R298K Top SDM	GCCTACGCCTAAGACGCCAAAACAGTTGCGTGAAT TTTTGGGCACAG
91	MMLV R298E Top SDM	GCCTACGCCTAAGACGCCAGAACAGTTGCGTGAAT TTTTGGGCACAG
92	MMLV R301A Top SDM	CCTAAGACGCCACGCCAGTTGGCGGAATTTTGGG CACAGCGGGA
93	MMLV R301K Top SDM	CCTAAGACGCCACGCCAGTTGAAAGAATTTTGGG CACAGCGGGA

94	MMLV R301E Top SDM	CCTAAGACGCCACGCCAGTTGGAAGAATTTTGGG CACAGCGGGA
95	MMLV K329A Top SDM	GCACCCCTGTACCCCTTAACAGCGACAGGGACGCT TTTCAACTGG
96	MMLV K329R Top SDM	GCACCCCTGTACCCCTTAACACGTACAGGGACGCT TTTCAACTGG
97	MMLV K329E Top SDM	GCACCCCTGTACCCCTTAACAGAAACAGGGACGCT TTTCAACTGG
98	MMLV K53A Btm SDM	GTTTGATAGAGACAGGTGTAGACGTTGCCGCTAAC GGGATGATCAACGGTGCTT
99	MMLV K53R Btm SDM	GTTTGATAGAGACAGGTGTAGACGTTGCACGTAAC GGGATGATCAACGGTGCTT
100	MMLV K53E Btm SDM	GTTTGATAGAGACAGGTGTAGACGTTGCTTCTAAC GGGATGATCAACGGTGCTT
101	MMLV T55A Btm SDM	GGGGTACTGTTTGATAGAGACAGGTGTAGACGCTG CCTTTAACGGGATGATCAACGG
102	MMLV T55R Btm SDM	GGGGTACTGTTTGATAGAGACAGGTGTAGAACGTG CCTTTAACGGGATGATCAACGG
103	MMLV T55E Btm SDM	GGGGTACTGTTTGATAGAGACAGGTGTAGATTCTG CCTTTAACGGGATGATCAACGG
104	MMLV T57A Btm SDM	CTCATGGGGTACTGTTTGATAGAGACAGGCGCAGA CGTTGCCTTTAACGGGATGAT
105	MMLV T57R Btm SDM	CTCATGGGGTACTGTTTGATAGAGACAGGACGAGA CGTTGCCTTTAACGGGATGAT
106	MMLV T57E Btm SDM	CTCATGGGGTACTGTTTGATAGAGACAGGTTTCAGA CGTTGCCTTTAACGGGATGAT
107	MMLV V59A Btm SDM	CCTCTTGACTCATGGGGTACTGTTTGATAGACGCA GGTGTAGACGTTGCCTTTAACGG
108	MMLV V59R Btm SDM	CCTCTTGACTCATGGGGTACTGTTTGATAGAACGA GGTGTAGACGTTGCCTTTAACGG
109	MMLV V59E Btm SDM	CCTCTTGACTCATGGGGTACTGTTTGATAGATTCA GGTGTAGACGTTGCCTTTAACGG
110	MMLV I61A Btm SDM	CCTCTTGACTCATGGGGTACTGTTTCGAGAGACA GGTGTAGACGTTGCCTTTA
111	MMLV I61R Btm SDM	CCTCTTGACTCATGGGGTACTGTTTACGAGAGACA GGTGTAGACGTTGCCTTTA
112	MMLV I61E Btm SDM	CCTCTTGACTCATGGGGTACTGTTTTTCAGAGACA GGTGTAGACGTTGCCTTTA
113	MMLV K62A Btm SDM	GCCTCTTGACTCATGGGGTACTGCGCGATAGAGAC AGGTGTAGACGTTGCC
114	MMLV K62R Btm SDM	GCCTCTTGACTCATGGGGTACTGACGGATAGAGAC AGGTGTAGACGTTGCC
115	MMLV K62E Btm	GCCTCTTGACTCATGGGGTACTGTTTCGATAGAGAC

	SDM	AGGTGTAGACGTTGCC
116	MMLV Q68A Btm SDM	CTGTCTCTATCAAACAGTACCCCATGAGTGCGGAG GCCCCGCTGGG
117	MMLV Q68R Btm SDM	CTGTCTCTATCAAACAGTACCCCATGAGTCGTGAG GCCCCGCTGGG
118	MMLV Q68E Btm SDM	CTGTCTCTATCAAACAGTACCCCATGAGTGAAGAG GCCCCGCTGGG
119	MMLV K75A Btm SDM	TGGTCCAGCAAGCGCTGAATATGTGGCGCAATCCC CAGGCGGGCC
120	MMLV K75R Btm SDM	TGGTCCAGCAAGCGCTGAATATGTGGACGAATCCC CAGGCGGGCC
121	MMLV K75E Btm SDM	TGGTCCAGCAAGCGCTGAATATGTGGTTCAATCCC CAGGCGGGCC
122	MMLV Q79A Btm SDM	CCCCTGGTCCAGCAAGCGCGCAATATGTGGCTTAA TCCCCAGGCG
123	MMLV Q79R Btm SDM	CCCCTGGTCCAGCAAGCGACGAATATGTGGCTTAA TCCCCAGGCG
124	MMLV Q79E Btm SDM	CCCCTGGTCCAGCAAGCGTTCAATATGTGGCTTAA TCCCCAGGCG
125	MMLV L99A Btm SDM	GTTTGTACCTGGCTTTTTCACGGGCGCAAGGGGGG TGTTCCACGG
126	MMLV L99R Btm SDM	GTTTGTACCTGGCTTTTTCACGGGACGAAGGGGGG TGTTCCACGG
127	MMLV L99E Btm SDM	GTTTGTACCTGGCTTTTTCACGGGTTCAAGGGGGG TGTTCCACGG
128	MMLV V101A Btm SDM	GGACGATAATCGTTTGTACCTGGCTTTTTTCGCGGG CAGAAGGGGGGTG
129	MMLV V101R Btm SDM	GGACGATAATCGTTTGTACCTGGCTTTTTTACGGGG CAGAAGGGGGGTG
130	MMLV V101E Btm SDM	GGACGATAATCGTTTGTACCTGGCTTTTTTTCGGG CAGAAGGGGGGTG
131	MMLV K102A Btm SDM	GGACGATAATCGTTTGTACCTGGCTTCGCCACGGG CAGAAGGGGG
132	MMLV K102R Btm SDM	GGACGATAATCGTTTGTACCTGGCTTACGCACGGG CAGAAGGGGG
133	MMLV K102E Btm SDM	GGACGATAATCGTTTGTACCTGGCTTTTCCACGGG CAGAAGGGGG
134	MMLV K103A Btm SDM	AAC TGGACGATAATCGTTTGTACCTGGCGCTTTCA CGGGCAGAAGGGGG
135	MMLV K103R Btm SDM	AAC TGGACGATAATCGTTTGTACCTGGACGTTTCA CGGGCAGAAGGGGG
136	MMLV K103E Btm SDM	AAC TGGACGATAATCGTTTGTACCTGGTTCTTTCA CGGGCAGAAGGGGG

137	MMLV T106A Btm SDM	CGAAGATCTTGAAGTGGACGATAATCGTTTCGCACC TGGCTTTTTTCACGGGC
138	MMLV T106R Btm SDM	CGAAGATCTTGAAGTGGACGATAATCGTTACGACC TGGCTTTTTTCACGGGC
139	MMLV T106E Btm SDM	CGAAGATCTTGAAGTGGACGATAATCGTTTTACACC TGGCTTTTTTCACGGGC
140	MMLV N107A Btm SDM	CGCGAAGATCTTGAAGTGGACGATAATCCGCTGTA CCTGGCTTTTTTCACGGG
141	MMLV N107R Btm SDM	CGCGAAGATCTTGAAGTGGACGATAATCACGTGTA CCTGGCTTTTTTCACGGG
142	MMLV N107E Btm SDM	CGCGAAGATCTTGAAGTGGACGATAATCTTCTGTA CCTGGCTTTTTTCACGGG
143	MMLV Y109A Btm SDM	CGCGAAGATCTTGAAGTGGACGCGCATCGTTTGTA CCTGGCTTTTTTCACG
144	MMLV Y109R Btm SDM	CGCGAAGATCTTGAAGTGGACGACGATCGTTTGTA CCTGGCTTTTTTCACG
145	MMLV Y109E Btm SDM	CGCGAAGATCTTGAAGTGGACGTTTCATCGTTTGTA CCTGGCTTTTTTCACG
146	MMLV R110A Btm SDM	CCTCGCGAAGATCTTGAAGTGGCGCATAATCGTTT GTACCTGGCTTTTTTCACG
147	MMLV R110K Btm SDM	CCTCGCGAAGATCTTGAAGTGGTTTATAATCGTTT GTACCTGGCTTTTTTCACG
148	MMLV R110E Btm SDM	CCTCGCGAAGATCTTGAAGTGGTTCATAATCGTTT GTACCTGGCTTTTTTCACG
149	MMLV V112A Btm SDM	GTTTGTTGACCTCGCGAAGATCTTGCGCTGGACGA TAATCGTTTGTACCTGGC
150	MMLV V112R Btm SDM	GTTTGTTGACCTCGCGAAGATCTTGACGTGGACGA TAATCGTTTGTACCTGGC
151	MMLV V112E Btm SDM	GTTTGTTGACCTCGCGAAGATCTTGTTCTGGACGA TAATCGTTTGTACCTGGC
152	MMLV K120A Btm SDM	GTCGGATGGATGTCTTCTACGCGCGCGTTGACCTC GCGAAGATCTTGAAGT
153	MMLV K120R Btm SDM	GTCGGATGGATGTCTTCTACGCGACGGTTGACCTC GCGAAGATCTTGAAGT
154	MMLV K120E Btm SDM	GTCGGATGGATGTCTTCTACGCGTTCGTTGACCTC GCGAAGATCTTGAAGT
155	MMLV E123A Btm SDM	GGATTAGGTACAGTCGGATGGATGTCCGCTACGCG TTTGTTGACCTCGC
156	MMLV E123R Btm SDM	GGATTAGGTACAGTCGGATGGATGTCACGTACGCG TTTGTTGACCTCGC
157	MMLV E123D Btm SDM	GGATTAGGTACAGTCGGATGGATGTCATCTACGCG TTTGTTGACCTCGC
158	MMLV T128V Btm	GCAGGCCTGATAACAGATTATAAGGATTAGGTACC

	SDM	ACCGGATGGATGTCTTCTACGCGT
159	MMLV T128R Btm SDM	GCAGGCCTGATAACAGATTATAAGGATTAGGTACA CGCGGATGGATGTCTTCTACGCGT
160	MMLV T128E Btm SDM	GCAGGCCTGATAACAGATTATAAGGATTAGGTACT TCCGGATGGATGTCTTCTACGCGT
161	MMLV K193A Btm SDM	TTCATCGAACAATGTGGGGCTGTTCGCAAAGCCCT GGGGCAGACG
162	MMLV K193R Btm SDM	TTCATCGAACAATGTGGGGCTGTTACGAAAGCCCT GGGGCAGACG
163	MMLV K193E Btm SDM	TTCATCGAACAATGTGGGGCTGTTTTCAAAGCCCT GGGGCAGACG
164	MMLV E282A Btm SDM	CATTACGGTCTCCTTACGCGCCGAGTCAGCCAAC GTTGACCTTCT
165	MMLV E282R Btm SDM	CATTACGGTCTCCTTACGCGCACGAGTCAGCCAAC GTTGACCTTCT
166	MMLV E282D Btm SDM	CATTACGGTCTCCTTACGCGCATCAGTCAGCCAAC GTTGACCTTCT
167	MMLV A283V Btm SDM	GCCCCATTACGGTCTCCTTACGCACTTCAGTCAGC CAACGTTGACCTTC
168	MMLV A283R Btm SDM	GCCCCATTACGGTCTCCTTACGACGTTTCAGTCAGC CAACGTTGACCTTC
169	MMLV A283E Btm SDM	GCCCCATTACGGTCTCCTTACGTTCTTCAGTCAGC CAACGTTGACCTTC
170	MMLV Q291A Btm SDM	CGTGGCGTCTTAGGCGTAGGCGCCCCATTACGGT CTCCTTACGC
171	MMLV Q291R Btm SDM	CGTGGCGTCTTAGGCGTAGGACGCCCCATTACGGT CTCCTTACGC
172	MMLV Q291E Btm SDM	CGTGGCGTCTTAGGCGTAGGTTCCCCCATTACGGT CTCCTTACGC
173	MMLV T293A Btm SDM	CAACTGGCGTGGCGTCTTAGGCGCAGGCTGCCCCA TTACGGTCTC
174	MMLV T293R Btm SDM	CAACTGGCGTGGCGTCTTAGGACGAGGCTGCCCCA TTACGGTCTC
175	MMLV T293E Btm SDM	CAACTGGCGTGGCGTCTTAGGTTTCAGGCTGCCCCA TTACGGTCTC
176	MMLV K295A Btm SDM	TTCACGCAACTGGCGTGGCGTCGCAGGCGTAGGCT GCCCCATTAC
177	MMLV K295R Btm SDM	TTCACGCAACTGGCGTGGCGTACGAGGCGTAGGCT GCCCCATTAC
178	MMLV K295E Btm SDM	TTCACGCAACTGGCGTGGCGTTTCAGGCGTAGGCT GCCCCATTAC
179	MMLV T296A Btm SDM	AAAATTACGCAACTGGCGTGGCGCCTTAGGCGTA GGCTGCCCCA

180	MMLV T296R Btm SDM	AAAATTACGCAACTGGCGTGGACGCTTAGGCGTA GGCTGCCCCA
181	MMLV T296E Btm SDM	AAAATTACGCAACTGGCGTGGTTCCTTAGGCGTA GGCTGCCCCA
182	MMLV R298A Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGCGCTGGCGTC TTAGGCGTAGGC
183	MMLV R298K Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGTTTTGGCGTC TTAGGCGTAGGC
184	MMLV R298E Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGTTCTGGCGTC TTAGGCGTAGGC
185	MMLV R301A Btm SDM	TCCCGCTGTGCCCCAAAAATTCGCGCAACTGGCGTG GCGTCTTAGG
186	MMLV R301K Btm SDM	TCCCGCTGTGCCCCAAAAATTCCTTCAACTGGCGTG GCGTCTTAGG
187	MMLV R301E Btm SDM	TCCCGCTGTGCCCCAAAAATTCCTCCAACCTGGCGTG GCGTCTTAGG
188	MMLV K329A Btm SDM	CCAGTTGAAAAGCGTCCCTGTCGCTGTTAAGGGGT ACAGGGGTGC
189	MMLV K329R Btm SDM	CCAGTTGAAAAGCGTCCCTGTACGTGTTAAGGGGT ACAGGGGTGC
190	MMLV K329E Btm SDM	CCAGTTGAAAAGCGTCCCTGTTTCTGTTAAGGGGT ACAGGGGTGC
191	MMLV I61G Top SDM	TAAAGGCAACGTCTACACCTGTCTCTGGCAAACAG TACCCCATGAGTCAAGAGG
192	MMLV I61G Btm SDM	CCTCTTGACTCATGGGGTACTGTTTGCCAGAGACA GGTGTAGACGTTGCCTTTA
193	MMLV I61L Top SDM	TAAAGGCAACGTCTACACCTGTCTCTCTGAAACAG TACCCCATGAGTCAAGAGG
194	MMLV I61L Btm SDM	CCTCTTGACTCATGGGGTACTGTTTCAGAGAGACA GGTGTAGACGTTGCCTTTA
195	MMLV I61V Top SDM	TAAAGGCAACGTCTACACCTGTCTCTGTGAAACAG TACCCCATGAGTCAAGAGG
196	MMLV I61V Btm SDM	CCTCTTGACTCATGGGGTACTGTTTCACAGAGACA GGTGTAGACGTTGCCTTTA
197	MMLV I61P Top SDM	TAAAGGCAACGTCTACACCTGTCTCTCCGAAACAG TACCCCATGAGTCAAGAGG
198	MMLV I61P Btm SDM	CCTCTTGACTCATGGGGTACTGTTTCGAGAGAGACA GGTGTAGACGTTGCCTTTA
199	MMLV I61M Top SDM	TAAAGGCAACGTCTACACCTGTCTCTATGAAACAG TACCCCATGAGTCAAGAGG
200	MMLV I61M Btm SDM	CCTCTTGACTCATGGGGTACTGTTTCATAGAGACA GGTGTAGACGTTGCCTTTA

201	MMLV I61S Top SDM	TAAAGGCAACGTCTACACCTGTCTCTAGCAAACAG TACCCCATGAGTCAAGAGG
202	MMLV I61S Btm SDM	CCTCTTGACTCATGGGGTACTGTTTGCTAGAGACA GGTGTAGACGTTGCCTTTA
203	MMLV I61T Top SDM	TAAAGGCAACGTCTACACCTGTCTCTACCAAACAG TACCCCATGAGTCAAGAGG
204	MMLV I61T Btm SDM	CCTCTTGACTCATGGGGTACTGTTTGCTAGAGACA GGTGTAGACGTTGCCTTTA
205	MMLV I61C Top SDM	TAAAGGCAACGTCTACACCTGTCTCTTGCAAACAG TACCCCATGAGTCAAGAGG
206	MMLV I61C Btm SDM	CCTCTTGACTCATGGGGTACTGTTTGCAAGAGACA GGTGTAGACGTTGCCTTTA
207	MMLV I61F Top SDM	TAAAGGCAACGTCTACACCTGTCTCTTTTAAACAG TACCCCATGAGTCAAGAGG
208	MMLV I61F Btm SDM	CCTCTTGACTCATGGGGTACTGTTTAAAAGAGACA GGTGTAGACGTTGCCTTTA
209	MMLV I61Y Top SDM	TAAAGGCAACGTCTACACCTGTCTCTTATAAACAG TACCCCATGAGTCAAGAGG
210	MMLV I61Y Btm SDM	CCTCTTGACTCATGGGGTACTGTTTATAAGAGACA GGTGTAGACGTTGCCTTTA
211	MMLV I61H Top SDM	TAAAGGCAACGTCTACACCTGTCTCTCATAAACAG TACCCCATGAGTCAAGAGG
212	MMLV I61H Btm SDM	CCTCTTGACTCATGGGGTACTGTTTATGAGAGACA GGTGTAGACGTTGCCTTTA
213	MMLV I61W Top SDM	TAAAGGCAACGTCTACACCTGTCTCTTGAAACAG TACCCCATGAGTCAAGAGG
214	MMLV I61W Btm SDM	CCTCTTGACTCATGGGGTACTGTTTCCAAGAGACA GGTGTAGACGTTGCCTTTA
215	MMLV I61D Top SDM	TAAAGGCAACGTCTACACCTGTCTCTGATAAACAG TACCCCATGAGTCAAGAGG
216	MMLV I61D Btm SDM	CCTCTTGACTCATGGGGTACTGTTTATCAGAGACA GGTGTAGACGTTGCCTTTA
217	MMLV I61N Top SDM	TAAAGGCAACGTCTACACCTGTCTCTAACAAACAG TACCCCATGAGTCAAGAGG
218	MMLV I61N Btm SDM	CCTCTTGACTCATGGGGTACTGTTTGTTAGAGACA GGTGTAGACGTTGCCTTTA
219	MMLV I61Q Top SDM	TAAAGGCAACGTCTACACCTGTCTCTCAGAAACAG TACCCCATGAGTCAAGAGG
220	MMLV I61Q Btm SDM	CCTCTTGACTCATGGGGTACTGTTTCTGAGAGACA GGTGTAGACGTTGCCTTTA
221	MMLV I61K Top SDM	TAAAGGCAACGTCTACACCTGTCTCTAAAAAACAG TACCCCATGAGTCAAGAGG
222	MMLV I61K Btm SDM	CCTCTTGACTCATGGGGTACTGTTTTTTAGAGACA

	SDM	GGTGTAGACGTTGCCTTTA
223	MMLV Q68G Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTGGCGAG GCCCCGCTGGG
224	MMLV Q68G Btm SDM	CCCAGGCGGGCCTCGCCACTCATGGGGTACTGTTT GATAGAGACAG
225	MMLV Q68L Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTCTGGAG GCCCCGCTGGG
226	MMLV Q68L Btm SDM	CCCAGGCGGGCCTCCAGACTCATGGGGTACTGTTT GATAGAGACAG
227	MMLV Q68I Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTATTGAG GCCCCGCTGGG
228	MMLV Q68I Btm SDM	CCCAGGCGGGCCTCAATACTCATGGGGTACTGTTT GATAGAGACAG
229	MMLV Q68V Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTGTGGAG GCCCCGCTGGG
230	MMLV Q68V Btm SDM	CCCAGGCGGGCCTCCACACTCATGGGGTACTGTTT GATAGAGACAG
231	MMLV Q68P Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTCCGGAG GCCCCGCTGGG
232	MMLV Q68P Btm SDM	CCCAGGCGGGCCTCCGGACTCATGGGGTACTGTTT GATAGAGACAG
233	MMLV Q68M Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTATGGAG GCCCCGCTGGG
234	MMLV Q68M Btm SDM	CCCAGGCGGGCCTCCATACTCATGGGGTACTGTTT GATAGAGACAG
235	MMLV Q68S Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTAGCGAG GCCCCGCTGGG
236	MMLV Q68S Btm SDM	CCCAGGCGGGCCTCGCTACTCATGGGGTACTGTTT GATAGAGACAG
237	MMLV Q68T Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTACCGAG GCCCCGCTGGG
238	MMLV Q68T Btm SDM	CCCAGGCGGGCCTCGGTACTCATGGGGTACTGTTT GATAGAGACAG
239	MMLV Q68C Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTTGCGAG GCCCCGCTGGG
240	MMLV Q68C Btm SDM	CCCAGGCGGGCCTCGCAACTCATGGGGTACTGTTT GATAGAGACAG
241	MMLV Q68F Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTTTGTAG GCCCCGCTGGG
242	MMLV Q68F Btm SDM	CCCAGGCGGGCCTCAAACTCATGGGGTACTGTTT GATAGAGACAG
243	MMLV Q68Y Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTTATGAG GCCCCGCTGGG

244	MMLV Q68Y Btm SDM	CCCAGGCGGGCCTCATAACTCATGGGGTACTGTTT GATAGAGACAG
245	MMLV Q68H Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTCATGAG GCCCCGCTGGG
246	MMLV Q68H Btm SDM	CCCAGGCGGGCCTCATGACTCATGGGGTACTGTTT GATAGAGACAG
247	MMLV Q68W Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTTGGGAG GCCCCGCTGGG
248	MMLV Q68W Btm SDM	CCCAGGCGGGCCTCCCAACTCATGGGGTACTGTTT GATAGAGACAG
249	MMLV Q68D Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTGATGAG GCCCCGCTGGG
250	MMLV Q68D Btm SDM	CCCAGGCGGGCCTCATCACTCATGGGGTACTGTTT GATAGAGACAG
251	MMLV Q68N Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTAACGAG GCCCCGCTGGG
252	MMLV Q68N Btm SDM	CCCAGGCGGGCCTCGTTACTCATGGGGTACTGTTT GATAGAGACAG
253	MMLV Q68K Top SDM	CTGTCTCTATCAAACAGTACCCCATGAGTAAAGAG GCCCCGCTGGG
254	MMLV Q68K Btm SDM	CCCAGGCGGGCCTCTTTACTCATGGGGTACTGTTT GATAGAGACAG
255	MMLV Q79G Top SDM	CGCCTGGGGATTAAGCCACATATTGGCCGCTTGCT GGACCAGGGG
256	MMLV Q79G Btm SDM	CCCCTGGTCCAGCAAGCGGCAATATGTGGCTTAA TCCCCAGGCG
257	MMLV Q79L Top SDM	CGCCTGGGGATTAAGCCACATATTCTGCGCTTGCT GGACCAGGGG
258	MMLV Q79L Btm SDM	CCCCTGGTCCAGCAAGCGCAGAATATGTGGCTTAA TCCCCAGGCG
259	MMLV Q79I Top SDM	CGCCTGGGGATTAAGCCACATATTATTCGCTTGCT GGACCAGGGG
260	MMLV Q79I Btm SDM	CCCCTGGTCCAGCAAGCGAATAATATGTGGCTTAA TCCCCAGGCG
261	MMLV Q79V Top SDM	CGCCTGGGGATTAAGCCACATATTGTGCGCTTGCT GGACCAGGGG
262	MMLV Q79V Btm SDM	CCCCTGGTCCAGCAAGCGCACAATATGTGGCTTAA TCCCCAGGCG
263	MMLV Q79P Top SDM	CGCCTGGGGATTAAGCCACATATTCCGCGCTTGCT GGACCAGGGG
264	MMLV Q79P Btm SDM	CCCCTGGTCCAGCAAGCGCGGAATATGTGGCTTAA TCCCCAGGCG
265	MMLV Q79M Top	CGCCTGGGGATTAAGCCACATATTATGCGCTTGCT

	SDM	GGACCAGGGG
266	MMLV Q79M Btm SDM	CCCCTGGTCCAGCAAGCGCATAATATGTGGCTTAA TCCCCAGGCG
267	MMLV Q79S Top SDM	CGCCTGGGGATTAAGCCACATATTAGCCGCTTGCT GGACCAGGGG
268	MMLV Q79S Btm SDM	CCCCTGGTCCAGCAAGCGGCTAATATGTGGCTTAA TCCCCAGGCG
269	MMLV Q79T Top SDM	CGCCTGGGGATTAAGCCACATATTACCCGCTTGCT GGACCAGGGG
270	MMLV Q79T Btm SDM	CCCCTGGTCCAGCAAGCGGGTAATATGTGGCTTAA TCCCCAGGCG
271	MMLV Q79C Top SDM	CGCCTGGGGATTAAGCCACATATTTGCCGCTTGCT GGACCAGGGG
272	MMLV Q79C Btm SDM	CCCCTGGTCCAGCAAGCGGCAAATATGTGGCTTAA TCCCCAGGCG
273	MMLV Q79F Top SDM	CGCCTGGGGATTAAGCCACATATTTTTTCGCTTGCT GGACCAGGGG
274	MMLV Q79F Btm SDM	CCCCTGGTCCAGCAAGCGAAAAATATGTGGCTTAA TCCCCAGGCG
275	MMLV Q79Y Top SDM	CGCCTGGGGATTAAGCCACATATTTATCGCTTGCT GGACCAGGGG
276	MMLV Q79Y Btm SDM	CCCCTGGTCCAGCAAGCGATAAATATGTGGCTTAA TCCCCAGGCG
277	MMLV Q79H Top SDM	CGCCTGGGGATTAAGCCACATATTCATCGCTTGCT GGACCAGGGG
278	MMLV Q79H Btm SDM	CCCCTGGTCCAGCAAGCGATGAATATGTGGCTTAA TCCCCAGGCG
279	MMLV Q79W Top SDM	CGCCTGGGGATTAAGCCACATATTTGGCGCTTGCT GGACCAGGGG
280	MMLV Q79W Btm SDM	CCCCTGGTCCAGCAAGCGCCAAATATGTGGCTTAA TCCCCAGGCG
281	MMLV Q79D Top SDM	CGCCTGGGGATTAAGCCACATATTGATCGCTTGCT GGACCAGGGG
282	MMLV Q79D Btm SDM	CCCCTGGTCCAGCAAGCGATCAATATGTGGCTTAA TCCCCAGGCG
283	MMLV Q79N Top SDM	CGCCTGGGGATTAAGCCACATATTAACCGCTTGCT GGACCAGGGG
284	MMLV Q79N Btm SDM	CCCCTGGTCCAGCAAGCGGTTAATATGTGGCTTAA TCCCCAGGCG
285	MMLV Q79K Top SDM	CGCCTGGGGATTAAGCCACATATTAAACGCTTGCT GGACCAGGGG
286	MMLV Q79K Btm SDM	CCCCTGGTCCAGCAAGCGTTTAATATGTGGCTTAA TCCCCAGGCG

287	MMLV L99G Top SDM	CCGTGGAACACCCCCCTTGGCCCCGTGAAAAAGCC AGGTACAAAC
288	MMLV L99G Btm SDM	GTTTGTACCTGGCTTTTTCACGGGGCCAAGGGGGG TGTTCCACGG
289	MMLV L99I Top SDM	CCGTGGAACACCCCCCTTATTCCCGTGAAAAAGCC AGGTACAAAC
290	MMLV L99I Btm SDM	GTTTGTACCTGGCTTTTTCACGGGAATAAGGGGGG TGTTCCACGG
291	MMLV L99V Top SDM	CCGTGGAACACCCCCCTTGTGCCCCGTGAAAAAGCC AGGTACAAAC
292	MMLV L99V Btm SDM	GTTTGTACCTGGCTTTTTCACGGGCACAAGGGGGG TGTTCCACGG
293	MMLV L99P Top SDM	CCGTGGAACACCCCCCTTCCGCCCCGTGAAAAAGCC AGGTACAAAC
294	MMLV L99P Btm SDM	GTTTGTACCTGGCTTTTTCACGGGCGGAAGGGGGG TGTTCCACGG
295	MMLV L99M Top SDM	CCGTGGAACACCCCCCTTATGCCCCGTGAAAAAGCC AGGTACAAAC
296	MMLV L99M Btm SDM	GTTTGTACCTGGCTTTTTCACGGGCATAAGGGGGG TGTTCCACGG
297	MMLV L99S Top SDM	CCGTGGAACACCCCCCTTAGCCCCGTGAAAAAGCC AGGTACAAAC
298	MMLV L99S Btm SDM	GTTTGTACCTGGCTTTTTCACGGGGCTAAGGGGGG TGTTCCACGG
299	MMLV L99T Top SDM	CCGTGGAACACCCCCCTTACCCCCGTGAAAAAGCC AGGTACAAAC
300	MMLV L99T Btm SDM	GTTTGTACCTGGCTTTTTCACGGGGGTAAGGGGGG TGTTCCACGG
301	MMLV L99C Top SDM	CCGTGGAACACCCCCCTTTGCCCCGTGAAAAAGCC AGGTACAAAC
302	MMLV L99C Btm SDM	GTTTGTACCTGGCTTTTTCACGGGGCAAAGGGGGG TGTTCCACGG
303	MMLV L99F Top SDM	CCGTGGAACACCCCCCTTTTTCCTCGTGAAAAAGCC AGGTACAAAC
304	MMLV L99F Btm SDM	GTTTGTACCTGGCTTTTTCACGGGAAAAAGGGGGG TGTTCCACGG
305	MMLV L99Y Top SDM	CCGTGGAACACCCCCCTTTATCCCGTGAAAAAGCC AGGTACAAAC
306	MMLV L99Y Btm SDM	GTTTGTACCTGGCTTTTTCACGGGATAAAGGGGGG TGTTCCACGG
307	MMLV L99H Top SDM	CCGTGGAACACCCCCCTTCATCCCGTGAAAAAGCC AGGTACAAAC
308	MMLV L99H Btm SDM	GTTTGTACCTGGCTTTTTCACGGGATGAAGGGGGG

	SDM	TGTTCCACGG
309	MMLV L99W Top SDM	CCGTGGAACACCCCCCTTTGGCCCGTGAAAAAGCC AGGTACAAAC
310	MMLV L99W Btm SDM	GTTTGTACCTGGCTTTTTCACGGGCCAAAGGGGGG TGTTCCACGG
311	MMLV L99D Top SDM	CCGTGGAACACCCCCCTTGATCCCGTGAAAAAGCC AGGTACAAAC
312	MMLV L99D Btm SDM	GTTTGTACCTGGCTTTTTCACGGGATCAAGGGGGG TGTTCCACGG
313	MMLV L99N Top SDM	CCGTGGAACACCCCCCTTAACCCCGTGAAAAAGCC AGGTACAAAC
314	MMLV L99N Btm SDM	GTTTGTACCTGGCTTTTTCACGGGGTTAAGGGGGG TGTTCCACGG
315	MMLV L99Q Top SDM	CCGTGGAACACCCCCCTTCAGCCCGTGAAAAAGCC AGGTACAAAC
316	MMLV L99Q Btm SDM	GTTTGTACCTGGCTTTTTCACGGGCTGAAGGGGGG TGTTCCACGG
317	MMLV L99K Top SDM	CCGTGGAACACCCCCCTTAAACCCGTGAAAAAGCC AGGTACAAAC
318	MMLV L99K Btm SDM	GTTTGTACCTGGCTTTTTCACGGGTTTAAGGGGGG TGTTCCACGG
319	MMLV E282G Top SDM	AGAAGGTCAACGTTGGCTGACTGGCGCGCGTAAGG AGACCGTAATG
320	MMLV E282G Btm SDM	CATTACGGTCTCCTTACGCGCGCCAGTCAGCCAAC GTTGACCTTCT
321	MMLV E282L Top SDM	AGAAGGTCAACGTTGGCTGACTCTGGCGCGTAAGG AGACCGTAATG
322	MMLV E282L Btm SDM	CATTACGGTCTCCTTACGCGCCAGAGTCAGCCAAC GTTGACCTTCT
323	MMLV E282I Top SDM	AGAAGGTCAACGTTGGCTGACTATTGCGCGTAAGG AGACCGTAATG
324	MMLV E282I Btm SDM	CATTACGGTCTCCTTACGCGCAATAGTCAGCCAAC GTTGACCTTCT
325	MMLV E282V Top SDM	AGAAGGTCAACGTTGGCTGACTGTGGCGCGTAAGG AGACCGTAATG
326	MMLV E282V Btm SDM	CATTACGGTCTCCTTACGCGCCACAGTCAGCCAAC GTTGACCTTCT
327	MMLV E282P Top SDM	AGAAGGTCAACGTTGGCTGACTCCGGCGCGTAAGG AGACCGTAATG
328	MMLV E282P Btm SDM	CATTACGGTCTCCTTACGCGCCGAGTCAGCCAAC GTTGACCTTCT
329	MMLV E282M Top SDM	AGAAGGTCAACGTTGGCTGACTATGGCGCGTAAGG AGACCGTAATG

330	MMLV E282M Btm SDM	CATTACGGTCTCCTTACGCGCCATAGTCAGCCAAC GTTGACCTTCT
331	MMLV E282S Top SDM	AGAAGGTCAACGTTGGCTGACTAGCGCGCGTAAGG AGACCGTAATG
332	MMLV E282S Btm SDM	CATTACGGTCTCCTTACGCGCGCTAGTCAGCCAAC GTTGACCTTCT
333	MMLV E282T Top SDM	AGAAGGTCAACGTTGGCTGACTACCGCGCGTAAGG AGACCGTAATG
334	MMLV E282T Btm SDM	CATTACGGTCTCCTTACGCGCGGTAGTCAGCCAAC GTTGACCTTCT
335	MMLV E282C Top SDM	AGAAGGTCAACGTTGGCTGACTTGCGCGCGTAAGG AGACCGTAATG
336	MMLV E282C Btm SDM	CATTACGGTCTCCTTACGCGCGCAAGTCAGCCAAC GTTGACCTTCT
337	MMLV E282F Top SDM	AGAAGGTCAACGTTGGCTGACTTTTGCGCGTAAGG AGACCGTAATG
338	MMLV E282F Btm SDM	CATTACGGTCTCCTTACGCGCAAAAGTCAGCCAAC GTTGACCTTCT
339	MMLV E282Y Top SDM	AGAAGGTCAACGTTGGCTGACTTATGCGCGTAAGG AGACCGTAATG
340	MMLV E282Y Btm SDM	CATTACGGTCTCCTTACGCGCATAAGTCAGCCAAC GTTGACCTTCT
341	MMLV E282H Top SDM	AGAAGGTCAACGTTGGCTGACTCATGCGCGTAAGG AGACCGTAATG
342	MMLV E282H Btm SDM	CATTACGGTCTCCTTACGCGCATGAGTCAGCCAAC GTTGACCTTCT
343	MMLV E282W Top SDM	AGAAGGTCAACGTTGGCTGACTTGGGCGCGTAAGG AGACCGTAATG
344	MMLV E282W Btm SDM	CATTACGGTCTCCTTACGCGCCCAAGTCAGCCAAC GTTGACCTTCT
345	MMLV E282N Top SDM	AGAAGGTCAACGTTGGCTGACTAACGCGCGTAAGG AGACCGTAATG
346	MMLV E282N Btm SDM	CATTACGGTCTCCTTACGCGCGTTAGTCAGCCAAC GTTGACCTTCT
347	MMLV E282Q Top SDM	AGAAGGTCAACGTTGGCTGACTCAGGCGCGTAAGG AGACCGTAATG
348	MMLV E282Q Btm SDM	CATTACGGTCTCCTTACGCGCCTGAGTCAGCCAAC GTTGACCTTCT
349	MMLV E282K Top SDM	AGAAGGTCAACGTTGGCTGACTAAAGCGCGTAAGG AGACCGTAATG
350	MMLV E282K Btm SDM	CATTACGGTCTCCTTACGCGCTTTAGTCAGCCAAC GTTGACCTTCT
351	MMLV R298G Top	GCCTACGCCTAAGACGCCAGGCCAGTTGCGTGAAT

	SDM	TTTTGGGCACAG
352	MMLV R298G Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGGCCTGGCGTC TTAGGCGTAGGC
353	MMLV R298L Top SDM	GCCTACGCCTAAGACGCCACTGCAGTTGCGTGAAT TTTTGGGCACAG
354	MMLV R298L Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGCAGTGGCGTC TTAGGCGTAGGC
355	MMLV R298I Top SDM	GCCTACGCCTAAGACGCCAATTCAGTTGCGTGAAT TTTTGGGCACAG
356	MMLV R298I Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGAATTGGCGTC TTAGGCGTAGGC
357	MMLV R298V Top SDM	GCCTACGCCTAAGACGCCAGTGCAGTTGCGTGAAT TTTTGGGCACAG
358	MMLV R298V Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGCACTGGCGTC TTAGGCGTAGGC
359	MMLV R298P Top SDM	GCCTACGCCTAAGACGCCACCGCAGTTGCGTGAAT TTTTGGGCACAG
360	MMLV R298P Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGCGGTGGCGTC TTAGGCGTAGGC
361	MMLV R298M Top SDM	GCCTACGCCTAAGACGCCAATGCAGTTGCGTGAAT TTTTGGGCACAG
362	MMLV R298M Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGCATTGGCGTC TTAGGCGTAGGC
363	MMLV R298S Top SDM	GCCTACGCCTAAGACGCCAAGCCAGTTGCGTGAAT TTTTGGGCACAG
364	MMLV R298S Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGGCTTGGCGTC TTAGGCGTAGGC
365	MMLV R298T Top SDM	GCCTACGCCTAAGACGCCAACCAGTTGCGTGAAT TTTTGGGCACAG
366	MMLV R298T Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGGGTGGCGTC TTAGGCGTAGGC
367	MMLV R298C Top SDM	GCCTACGCCTAAGACGCCATGCCAGTTGCGTGAAT TTTTGGGCACAG
368	MMLV R298C Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGGCATGGCGTC TTAGGCGTAGGC
369	MMLV R298F Top SDM	GCCTACGCCTAAGACGCCATTTAGTTGCGTGAAT TTTTGGGCACAG
370	MMLV R298F Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGAAATGGCGTC TTAGGCGTAGGC
371	MMLV R298Y Top SDM	GCCTACGCCTAAGACGCCATATCAGTTGCGTGAAT TTTTGGGCACAG
372	MMLV R298Y Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGATATGGCGTC TTAGGCGTAGGC

373	MMLV R298H Top SDM	GCCTACGCCTAAGACGCCACATCAGTTGCGTGAAT TTTTGGGCACAG
374	MMLV R298H Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGATGTGGCGTC TTAGGCGTAGGC
375	MMLV R298W Top SDM	GCCTACGCCTAAGACGCCATGGCAGTTGCGTGAAT TTTTGGGCACAG
376	MMLV R298W Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGCCATGGCGTC TTAGGCGTAGGC
377	MMLV R298D Top SDM	GCCTACGCCTAAGACGCCAGATCAGTTGCGTGAAT TTTTGGGCACAG
378	MMLV R298D Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGATCTGGCGTC TTAGGCGTAGGC
379	MMLV R298N Top SDM	GCCTACGCCTAAGACGCCAAACCAGTTGCGTGAAT TTTTGGGCACAG
380	MMLV R298N Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGGTTTGGCGTC TTAGGCGTAGGC
381	MMLV R298Q Top SDM	GCCTACGCCTAAGACGCCACAGCAGTTGCGTGAAT TTTTGGGCACAG
382	MMLV R298Q Btm SDM	CTGTGCCCCAAAAATTCACGCAACTGCTGTGGCGTC TTAGGCGTAGGC
383	MMLV I61R/Q68R Top SDM	AGGCAACGTCTACACCTGTCTCTCGTAAACAGTAC CCCATGAGTCGTGAGGCCCGCCTGGGG
384	MMLV I61R/Q68R Btm SDM	CCCCAGGCGGGCCTCACGACTCATGGGGTACTGTT TACGAGAGACAGGTGTAGACGTTGCCT
385	MMLV I61K/Q68R Top SDM	AGGCAACGTCTACACCTGTCTCTAAAAAACAGTAC CCCATGAGTCGTGAGG
386	MMLV I61K/Q68R Btm SDM	CCTCACGACTCATGGGGTACTGTTTTTTAGAGACA GGTGTAGACGTTGCCT
387	MMLV I61M/Q68R Top SDM	AGGCAACGTCTACACCTGTCTCTATGAAACAGTAC CCCATGAGTCGTGAGG
388	MMLV I61M/Q68R Btm SDM	CCTCACGACTCATGGGGTACTGTTTCATAGAGACA GGTGTAGACGTTGCCT
389	MMLV I61M/Q68I Top SDM	AGGCAACGTCTACACCTGTCTCTATGAAACAGTAC CCCATGAGTATTGAGGCC
390	MMLV I61M/Q68I Btm SDM	GGCCTCAATACTCATGGGGTACTGTTTCATAGAGA CAGGTGTAGACGTTGCCT
393	MMLV 5' Primer	GTCTCTATCAAACAGTACCCCATGGCGCAAGAGGC CCGCCTGGG
394	MMLV 3' Primer	GTCTCTATCAAACAGTACCCCATGCGTCAAGAGGC CCGCCTGGG
395	MMLV G73A Top SDM	CATGAGTCAAGAGGCCCGCGAGGGGATTAAGCCAC ATATTCAGCG
396	MMLV G73R Top	GAGTCAAGAGGCCCGCCTGGCGATTAAGCCACATA

	SDM	TTCAGCGCTTGC
397	MMLV G73E Top SDM	GAGTCAAGAGGCCCCGCTGCGTATTAAGCCACATA TTCAGCGCTTGC
398	MMLV P76A Top SDM	GAGTCAAGAGGCCCCGCTGGAGATTAAGCCACATA TTCAGCGCTTGC
399	MMLV P76R Top SDM	GGCCCCGCTGGGGATTAAGGCGCATATTCAGCGCT TGCTGGACC
400	MMLV P76E Top SDM	GGCCCCGCTGGGGATTAAGCGTCATATTCAGCGCT TGCTGGACC
401	MMLV H77A Top SDM	GGCCCCGCTGGGGATTAAGGAGCATATTCAGCGCT TGCTGGACC
402	MMLV H77R Top SDM	CCGCCTGGGGATTAAGCCAGCGATTCAGCGCTTGC TGGACCAG
403	MMLV H77E Top SDM	CCGCCTGGGGATTAAGCCACGTATTCAGCGCTTGC TGGACCAG
404	MMLV L82A Top SDM	CCGCCTGGGGATTAAGCCAGAGATTCAGCGCTTGC TGGACCAG
405	MMLV L82R Top SDM	GATTAAGCCACATATTCAGCGCTTGGCGGACCAGG GGATCTTGGTCC
406	MMLV L82E Top SDM	GATTAAGCCACATATTCAGCGCTTGGCTGACCAGG GGATCTTGGTCC
407	MMLV D83A Top SDM	GATTAAGCCACATATTCAGCGCTTGGAGGACCAGG GGATCTTGGTCC
408	MMLV D83R Top SDM	GCCACATATTCAGCGCTTGCTGGCGCAGGGGATCT TGGTCCCATG
409	MMLV D83E Top SDM	GCCACATATTCAGCGCTTGCTGCGTCAGGGGATCT TGGTCCCATG
410	MMLV I125A Top SDM	GCCACATATTCAGCGCTTGCTGGAGCAGGGGATCT TGGTCCCATG
411	MMLV I125R Top SDM	AGGTCAACAAACGCGTAGAAGACGCGCATCCGACT GTACCTAATCCTTATAAT
412	MMLV I125E Top SDM	AGGTCAACAAACGCGTAGAAGACCGTCATCCGACT GTACCTAATCCTTATAAT
413	MMLV V129A Top SDM	AGGTCAACAAACGCGTAGAAGACGAGCATCCGACT GTACCTAATCCTTATAAT
414	MMLV V129R Top SDM	GCGTAGAAGACATCCATCCGACTGCGCCTAATCCT TATAATCTGTTATCAGGC
415	MMLV V129E Top SDM	GCGTAGAAGACATCCATCCGACTCGTCCTAATCCT TATAATCTGTTATCAGGC
416	MMLV L198A Top SDM	GCGTAGAAGACATCCATCCGACTGAGCCTAATCCT TATAATCTGTTATCAGGC
417	MMLV L198R Top SDM	AGGGCTTTAAAAACAGCCCCACAGCGTTCGATGAA GCACTTCACCGTGA

418	MMLV L198E Top SDM	AGGGCTTTAAAAACAGCCCCACACGTTTCGATGAA GCACTTCACCGTGA
419	MMLV E201A Top SDM	AGGGCTTTAAAAACAGCCCCACAGAGTTCGATGAA GCACTTCACCGTGA
420	MMLV E201R Top SDM	TTTAAAAACAGCCCCACATTGTTTCGATGCGGCACT TCACCGTGACTTAGCAG
421	MMLV E201D Top SDM	TTTAAAAACAGCCCCACATTGTTTCGATCGTGCACT TCACCGTGACTTAGCAG
422	MMLV R205A Top SDM	TTTAAAAACAGCCCCACATTGTTTCGATGATGCACT TCACCGTGACTTAGCAG
423	MMLV R205K Top SDM	CACATTGTTTCGATGAAGCACTTCACGCGGACTTAG CAGACTTCCGTATCCA
424	MMLV R205E Top SDM	CACATTGTTTCGATGAAGCACTTCACAAAGACTTAG CAGACTTCCGTATCCA
425	MMLV D209A Top SDM	GATGAAGCACTTCACCGTGACTTAGAGGACTTCCG TATCCAACACCCAG
426	MMLV D209R Top SDM	AAGCACTTCACCGTGACTTAGCAGCGTTCCGTATC CAACACCCAGACTT
427	MMLV D209E Top SDM	AAGCACTTCACCGTGACTTAGCACGTTTCCGTATC CAACACCCAGACTT
428	MMLV F210A Top SDM	AAGCACTTCACCGTGACTTAGCAGAGTTCCGTATC CAACACCCAGACTT
429	MMLV F210R Top SDM	CACCTTCACCGTGACTTAGCAGACGCGGTATCCAA CACCCAGACTTAATTC
430	MMLV F210E Top SDM	CACCTTCACCGTGACTTAGCAGACCGTCGTATCCAA CACCCAGACTTAATTC
431	MMLV R211A Top SDM	CACCTTCACCGTGACTTAGCAGACGAGCGTATCCAA CACCCAGACTTAATTC
432	MMLV R211K Top SDM	TTCACCGTGACTTAGCAGACTTCGCGATCCAACAC CCAGACTTAATTCTGTTA
433	MMLV R211E Top SDM	TTCACCGTGACTTAGCAGACTTCAAAATCCAACAC CCAGACTTAATTCTGTTA
434	MMLV I212A Top SDM	TTCACCGTGACTTAGCAGACTTCGAGATCCAACAC CCAGACTTAATTCTGTTA
435	MMLV I212R Top SDM	CCGTGACTTAGCAGACTTCCGTGCGCAACACCCAG ACTTAATTCTGTTACAG
436	MMLV I212E Top SDM	CCGTGACTTAGCAGACTTCCGTGCGCAACACCCAG ACTTAATTCTGTTACAG
437	MMLV Q213A Top SDM	CCGTGACTTAGCAGACTTCCGTGAGCAACACCCAG ACTTAATTCTGTTACAG
438	MMLV Q213R Top SDM	GTGACTTAGCAGACTTCCGTATCGCGCACCCAGAC TTAATTCTGTTACAGTAT
439	MMLV Q213E Top	GTGACTTAGCAGACTTCCGTATCCGTCACCCAGAC

	SDM	TTAATTCTGTTACAGTAT
440	MMLV K348A Top SDM	GTGACTTAGCAGACTTCCGTATCGAGCACCCAGAC TTAATTCTGTTACAGTAT
441	MMLV K348R Top SDM	AGCAAAAGGCGTATCAGGAGATCGCGCAAGCTTTG TTGACCGCACCC
442	MMLV K348E Top SDM	AGCAAAAGGCGTATCAGGAGATCCGTCAAGCTTTG TTGACCGCACCC
443	MMLV L352A Top SDM	AGCAAAAGGCGTATCAGGAGATCGAGCAAGCTTTG TTGACCGCACCC
444	MMLV L352R Top SDM	CGTATCAGGAGATCAAACAAGCTTTGGCGACCGCA CCCGCGTTGGG
445	MMLV L352E Top SDM	CGTATCAGGAGATCAAACAAGCTTTGCGTACCGCA CCCGCGTTGGG
446	MMLV K285A Top SDM	CGTATCAGGAGATCAAACAAGCTTTGGAGACCGCA CCCGCGTTGGG
447	MMLV K285R Top SDM	GTTGGCTGACTGAAGCGCGTGCGGAGACCGTAATG GGGCAGC
448	MMLV K285E Top SDM	GTTGGCTGACTGAAGCGCGTCGTGAGACCGTAATG GGGCAGC
449	MMLV Q299A Top SDM	GTTGGCTGACTGAAGCGCGTGAGGAGACCGTAATG GGGCAGC
450	MMLV Q299R Top SDM	TACGCCTAAGACGCCACGCGCGTTGCGTGAATTTT TGGGCACAGC
451	MMLV Q299E Top SDM	TACGCCTAAGACGCCACGCCGTTTTCGTGAATTTT TGGGCACAGC
452	MMLV G308A Top SDM	TACGCCTAAGACGCCACGCGAGTTGCGTGAATTTT TGGGCACAGC
453	MMLV G308R Top SDM	GCGTGAATTTTTTGGGCACAGCGCGGTTCTGTCGTT TATGGATTCTGGG
454	MMLV G308E Top SDM	GCGTGAATTTTTTGGGCACAGCGCGTTCTGTCGTT TATGGATTCTGGG
455	MMLV R311A Top SDM	GCGTGAATTTTTTGGGCACAGCGGAGTTCTGTCGTT TATGGATTCTGGG
456	MMLV R311K Top SDM	GGGCACAGCGGGATTCTGTGCGTTATGGATTCTG GGTTCGCTGA
457	MMLV R311E Top SDM	GGGCACAGCGGGATTCTGTAAATTATGGATTCTG GGTTCGCTGA
458	MMLV Y271A Top SDM	GGGCACAGCGGGATTCTGTGAGTTATGGATTCTG GGTTCGCTGA
459	MMLV Y271R Top SDM	GTCAAAAACAGGTAAAGTACCTTGGGGCGTTGCTG AAAGAAGGTCAACGTTGG
460	MMLV Y271E Top SDM	GTCAAAAACAGGTAAAGTACCTTGGGGCGTTGCTG AAAGAAGGTCAACGTTGG

461	MMLV L280A Top SDM	GTCAAAAACAGGTAAAGTACCTTGGGGAGTTGCTG AAAGAAGGTCAACGTTGG
462	MMLV L280R Top SDM	TGCTGAAAGAAGGTCAACGTTGGGCGACTGAAGCG CGTAAGGAGACC
463	MMLV L280E Top SDM	TGCTGAAAGAAGGTCAACGTTGGCGTACTGAAGCG CGTAAGGAGACC
464	MMLV L357A Top SDM	TGCTGAAAGAAGGTCAACGTTGGGAGACTGAAGCG CGTAAGGAGACC
465	MMLV L357R Top SDM	TTTGTTGACCGCACCCGCGGCGGGTCTTCCGGATT TAACCAAGCC
466	MMLV L357E Top SDM	TTTGTTGACCGCACCCGCGCGTGGTCTTCCGGATT TAACCAAGCC
467	MMLV T328A Top SDM	TTTGTTGACCGCACCCGCGGAGGGTCTTCCGGATT TAACCAAGCC
468	MMLV T328R Top SDM	CTGCACCCCTGTACCCCTTAGCGAAAACAGGGACG CTTTTCAACTGG
469	MMLV T328E Top SDM	CTGCACCCCTGTACCCCTTACGTAAAACAGGGACG CTTTTCAACTGG
470	MMLV G331A Top SDM	CTGCACCCCTGTACCCCTTAGAGAAAACAGGGACG CTTTTCAACTGG
471	MMLV G331R Top SDM	CCCCTGTACCCCTTAACAAAAACAGCGACGCTTTT CAACTGGGGGCC
472	MMLV G331E Top SDM	CCCCTGTACCCCTTAACAAAAACACGTACGCTTTT CAACTGGGGGCC
473	MMLV T332A Top SDM	CCCCTGTACCCCTTAACAAAAACAGAGACGCTTTT CAACTGGGGGCC
474	MMLV T332R Top SDM	CTGTACCCCTTAACAAAAACAGGGGCGCTTTTCAA CTGGGGGCCAGAC
475	MMLV T332E Top SDM	CTGTACCCCTTAACAAAAACAGGGCGTCTTTTCAA CTGGGGGCCAGAC
476	MMLV N335A Top SDM	CTGTACCCCTTAACAAAAACAGGGGAGCTTTTCAA CTGGGGGCCAGAC
477	MMLV N335R Top SDM	CCTTAACAAAAACAGGGACGCTTTTCGCGTGGGG CCAGACCAGCAA
478	MMLV N335E Top SDM	CCTTAACAAAAACAGGGACGCTTTTCGCTTGGGG CCAGACCAGCAA
479	MMLV E367A Top SDM	CTTCCGGATTTAACCAAGCCCTTTGCGCTGTTTCGT TGATGAAAAACAGGGATAT
480	MMLV E367R Top SDM	CTTCCGGATTTAACCAAGCCCTTTGCTCTGTTTCGT TGATGAAAAACAGGGATAT
481	MMLV E367D Top SDM	CTTCCGGATTTAACCAAGCCCTTTGATCTGTTTCGT TGATGAAAAACAGGGATAT
482	MMLV F369A Top	GATTTAACCAAGCCCTTTGAGCTGGCGGTTGATGA

	SDM	AAAACAGGGATATGCAAAAG
483	MMLV F369R Top SDM	GATTTAACCAAGCCCTTTGAGCTGCGTGTTGATGA AAAACAGGGATATGCAAAAG
484	MMLV F369E Top SDM	GATTTAACCAAGCCCTTTGAGCTGGAGGTTGATGA AAAACAGGGATATGCAAAAG
485	MMLV R389A Top SDM	CCCAAAAGTTAGGCCCGTGGGCGCGCCCTGTTGCT TACTTGAGTAA
486	MMLV R389K Top SDM	CCCAAAAGTTAGGCCCGTGGAAACGCCCTGTTGCT TACTTGAGTAA
487	MMLV R389E Top SDM	CCCAAAAGTTAGGCCCGTGGGAGCGCCCTGTTGCT TACTTGAGTAA
488	MMLV V433A Top SDM	AGTTGACGATGGGTCAACCCTTAGCGATCTTGGCT CCACATGCTGTAGA
489	MMLV V433R Top SDM	AGTTGACGATGGGTCAACCCTTACGTATCTTGGCT CCACATGCTGTAGA
490	MMLV V433E Top SDM	AGTTGACGATGGGTCAACCCTTAGAGATCTTGGCT CCACATGCTGTAGA
491	MMLV V476A Top SDM	GGATCGTGTACAATTTGGACCAGTTGCGGCTTTGA ATCCAGCTACTTTGCTTC
492	MMLV V476R Top SDM	GGATCGTGTACAATTTGGACCAGTTGCTGCTTTGA ATCCAGCTACTTTGCTTC
493	MMLV V476E Top SDM	GGATCGTGTACAATTTGGACCAGTTGAGGCTTTGA ATCCAGCTACTTTGCTTC
494	MMLV I593A Top SDM	CGTTATGCTTTTGCAACAGCGCATGCGCATGGCGA AATTTACCGCCGC
495	MMLV I593R Top SDM	CGTTATGCTTTTGCAACAGCGCATCGTCATGGCGA AATTTACCGCCGC
496	MMLV I593E Top SDM	CGTTATGCTTTTGCAACAGCGCATGAGCATGGCGA AATTTACCGCCGC
497	MMLV E596A Top SDM	GCAACAGCGCATATCCATGGCGCGATTTACCGCCG CCGTGGTC
498	MMLV E596R Top SDM	GCAACAGCGCATATCCATGGCCGTATTTACCGCCG CCGTGGTC
499	MMLV E596D Top SDM	GCAACAGCGCATATCCATGGCGATATTTACCGCCG CCGTGGTC
500	MMLV I597A Top SDM	CAACAGCGCATATCCATGGCGAAGCGTACCGCCGC CGTGGTCTG
501	MMLV I597R Top SDM	CAACAGCGCATATCCATGGCGAACGTTACCGCCGC CGTGGTCTG
502	MMLV I597E Top SDM	CAACAGCGCATATCCATGGCGAAGAGTACCGCCGC CGTGGTCTG
503	MMLV R650A Top SDM	AGCGGAGGCTCGTGGAAACGCGATGGCGGACCAAG CTGCCC

504	MMLV R650K Top SDM	AGCGGAGGCTCGTGGAACAAAATGGCGGACCAAG CTGCCC
505	MMLV R650E Top SDM	AGCGGAGGCTCGTGGAACGAGATGGCGGACCAAG CTGCCC
506	MMLV Q654A Top SDM	GTGGAAACCGTATGGCGGACGCGGCTGCCCCGTAAG GCGGC
507	MMLV Q654R Top SDM	GTGGAAACCGTATGGCGGACCGTGCTGCCCCGTAAG GCGGC
508	MMLV Q654E Top SDM	GTGGAAACCGTATGGCGGACGAGGCTGCCCCGTAAG GCGGC
509	MMLV R657A Top SDM	TATGGCGGACCAAGCTGCCGCGAAGGCGGCGATCA CAGAGAC
510	MMLV R657K Top SDM	TATGGCGGACCAAGCTGCCAAAAGGCGGCGATCA CAGAGAC
511	MMLV R657E Top SDM	TATGGCGGACCAAGCTGCCGAGAAGGCGGCGATCA CAGAGAC
512	MMLV G73A Btm SDM	GCAAGCGCTGAATATGTGGCTTAATCGCCAGGCGG GCCTCTTGACTC
513	MMLV G73R Btm SDM	GCAAGCGCTGAATATGTGGCTTAATACGCAGGCGG GCCTCTTGACTC
514	MMLV G73E Btm SDM	GCAAGCGCTGAATATGTGGCTTAATCTCCAGGCGG GCCTCTTGACTC
515	MMLV P76A Btm SDM	GGTCCAGCAAGCGCTGAATATGCGCCTTAATCCCC AGGCGGGCC
516	MMLV P76R Btm SDM	GGTCCAGCAAGCGCTGAATATGACGCTTAATCCCC AGGCGGGCC
517	MMLV P76E Btm SDM	GGTCCAGCAAGCGCTGAATATGCTCCTTAATCCCC AGGCGGGCC
518	MMLV H77A Btm SDM	CTGGTCCAGCAAGCGCTGAATCGCTGGCTTAATCC CCAGGCGG
519	MMLV H77R Btm SDM	CTGGTCCAGCAAGCGCTGAATACGTGGCTTAATCC CCAGGCGG
520	MMLV H77E Btm SDM	CTGGTCCAGCAAGCGCTGAATCTCTGGCTTAATCC CCAGGCGG
521	MMLV L82A Btm SDM	GGACCAAGATCCCCCTGGTCCGCAAGCGCTGAATA TGTGGCTTAATC
522	MMLV L82R Btm SDM	GGACCAAGATCCCCCTGGTCACGCAAGCGCTGAATA TGTGGCTTAATC
523	MMLV L82E Btm SDM	GGACCAAGATCCCCCTGGTCCTCCAAGCGCTGAATA TGTGGCTTAATC
524	MMLV D83A Btm SDM	CATGGGACCAAGATCCCCCTGCGCCAGCAAGCGCTG AATATGTGGC
525	MMLV D83R Btm	CATGGGACCAAGATCCCCCTGACGCAGCAAGCGCTG

	SDM	AATATGTGGC
526	MMLV D83E Btm SDM	CATGGGACCAAGATCCCCTGCTCCAGCAAGCGCTG AATATGTGGC
527	MMLV I125A Btm SDM	ATTATAAGGATTAGGTACAGTCGGATGCGCGTCTT CTACGCGTTTGTTGACCT
528	MMLV I125R Btm SDM	ATTATAAGGATTAGGTACAGTCGGATGACGGTCTT CTACGCGTTTGTTGACCT
529	MMLV I125E Btm SDM	ATTATAAGGATTAGGTACAGTCGGATGCTCGTCTT CTACGCGTTTGTTGACCT
530	MMLV V129A Btm SDM	GCCTGATAACAGATTATAAGGATTAGGCGCAGTCG GATGGATGTCTTCTACGC
531	MMLV V129R Btm SDM	GCCTGATAACAGATTATAAGGATTAGGACGAGTCG GATGGATGTCTTCTACGC
532	MMLV V129E Btm SDM	GCCTGATAACAGATTATAAGGATTAGGCTCAGTCG GATGGATGTCTTCTACGC
533	MMLV L198A Btm SDM	TCACGGTGAAGTGCTTCATCGAACGCTGTGGGGCT GTTTTTAAAGCCCT
534	MMLV L198R Btm SDM	TCACGGTGAAGTGCTTCATCGAACGCTGTGGGGCT GTTTTTAAAGCCCT
535	MMLV L198E Btm SDM	TCACGGTGAAGTGCTTCATCGAACTCTGTGGGGCT GTTTTTAAAGCCCT
536	MMLV E201A Btm SDM	CTGCTAAGTCACGGTGAAGTGCCGCATCGAACAAT GTGGGGCTGTTTTTAAA
537	MMLV E201R Btm SDM	CTGCTAAGTCACGGTGAAGTGCACGATCGAACAAT GTGGGGCTGTTTTTAAA
538	MMLV E201D Btm SDM	CTGCTAAGTCACGGTGAAGTGCATCATCGAACAAT GTGGGGCTGTTTTTAAA
539	MMLV R205A Btm SDM	TGGATACGGAAGTCTGCTAAGTCCGCGTGAAGTGC TTCATCGAACAATGTG
540	MMLV R205K Btm SDM	TGGATACGGAAGTCTGCTAAGTCTTTGTGAAGTGC TTCATCGAACAATGTG
541	MMLV R205E Btm SDM	TGGATACGGAAGTCTGCTAAGTCCTCGTGAAGTGC TTCATCGAACAATGTG
542	MMLV D209A Btm SDM	AAGTCTGGGTGTTGGATACGGAACGCTGCTAAGTC ACGGTGAAGTGCTT
543	MMLV D209R Btm SDM	AAGTCTGGGTGTTGGATACGGAACGCTGCTAAGTC ACGGTGAAGTGCTT
544	MMLV D209E Btm SDM	AAGTCTGGGTGTTGGATACGGAACCTGCTAAGTC ACGGTGAAGTGCTT
545	MMLV F210A Btm SDM	GAATTAAGTCTGGGTGTTGGATACGCGCGTCTGCT AAGTCACGGTGAAGTG
546	MMLV F210R Btm SDM	GAATTAAGTCTGGGTGTTGGATACGACGGTCTGCT AAGTCACGGTGAAGTG

547	MMLV F210E Btm SDM	GAATTAAGTCTGGGTGTTGGATACGCTCGTCTGCT AAGTCACGGTGAAGTG
548	MMLV R211A Btm SDM	TAACAGAATTAAGTCTGGGTGTTGGATCGCGAAGT CTGCTAAGTCACGGTGAA
549	MMLV R211K Btm SDM	TAACAGAATTAAGTCTGGGTGTTGGATTTTGAAGT CTGCTAAGTCACGGTGAA
550	MMLV R211E Btm SDM	TAACAGAATTAAGTCTGGGTGTTGGATCTCGAAGT CTGCTAAGTCACGGTGAA
551	MMLV I212A Btm SDM	CTGTAACAGAATTAAGTCTGGGTGTTGCGCACGGA AGTCTGCTAAGTCACGG
552	MMLV I212R Btm SDM	CTGTAACAGAATTAAGTCTGGGTGTTGACGACGGA AGTCTGCTAAGTCACGG
553	MMLV I212E Btm SDM	CTGTAACAGAATTAAGTCTGGGTGTTGCTCACGGA AGTCTGCTAAGTCACGG
554	MMLV Q213A Btm SDM	ATACTGTAACAGAATTAAGTCTGGGTGCGCGATAC GGAAGTCTGCTAAGTCAC
555	MMLV Q213R Btm SDM	ATACTGTAACAGAATTAAGTCTGGGTGACGGATAC GGAAGTCTGCTAAGTCAC
556	MMLV Q213E Btm SDM	ATACTGTAACAGAATTAAGTCTGGGTGCTCGATAC GGAAGTCTGCTAAGTCAC
557	MMLV K348A Btm SDM	GGGTGCGGTCAACAAAGCTTGCGCGATCTCCTGAT ACGCCTTTTGCT
558	MMLV K348R Btm SDM	GGGTGCGGTCAACAAAGCTTGACGGATCTCCTGAT ACGCCTTTTGCT
559	MMLV K348E Btm SDM	GGGTGCGGTCAACAAAGCTTGCTCGATCTCCTGAT ACGCCTTTTGCT
560	MMLV L352A Btm SDM	CCCAACGCGGGTGCGGTGCGCAAAGCTTGTTTGAT CTCCTGATACG
561	MMLV L352R Btm SDM	CCCAACGCGGGTGCGGTACGCAAAGCTTGTTTGAT CTCCTGATACG
562	MMLV L352E Btm SDM	CCCAACGCGGGTGCGGTCTCCAAAGCTTGTTTGAT CTCCTGATACG
563	MMLV K285A Btm SDM	GCTGCCCCATTACGGTCTCCGCACGCGCTTCAGTC AGCCAAC
564	MMLV K285R Btm SDM	GCTGCCCCATTACGGTCTCACGACGCGCTTCAGTC AGCCAAC
565	MMLV K285E Btm SDM	GCTGCCCCATTACGGTCTCCTCACGCGCTTCAGTC AGCCAAC
566	MMLV Q299A Btm SDM	GCTGTGCCCAAAAATTCACGCAACGCGGTGGCGT CTTAGGCGTA
567	MMLV Q299R Btm SDM	GCTGTGCCCAAAAATTCACGCAACGCGGTGGCGT CTTAGGCGTA
568	MMLV Q299E	GCTGTGCCCAAAAATTCACGCAACTCGCGTGGCGT

	Btm SDM	CTTAGGCGTA
569	MMLV G308A Btm SDM	CCCAGGAATCCATAAACGACAGAACGCCGCTGTGC CCAAAAATTCACGC
570	MMLV G308R Btm SDM	CCCAGGAATCCATAAACGACAGAAACGCCGCTGTGC CCAAAAATTCACGC
571	MMLV G308E Btm SDM	CCCAGGAATCCATAAACGACAGAACTCCGCTGTGC CCAAAAATTCACGC
572	MMLV R311A Btm SDM	TCAGCGAACCCAGGAATCCATAACGCACAGAATCC CGCTGTGCCC
573	MMLV R311K Btm SDM	TCAGCGAACCCAGGAATCCATAATTTACAGAATCC CGCTGTGCCC
574	MMLV R311E Btm SDM	TCAGCGAACCCAGGAATCCATAACTCACAGAATCC CGCTGTGCCC
575	MMLV Y271A Btm SDM	CCAACGTTGACCTTCTTTCAGCAACGCCCCAAGGT ACTTTACCTGTTTTTGAC
576	MMLV Y271R Btm SDM	CCAACGTTGACCTTCTTTCAGCAAACGCCCCAAGGT ACTTTACCTGTTTTTGAC
577	MMLV Y271E Btm SDM	CCAACGTTGACCTTCTTTCAGCAACTCCCCAAGGT ACTTTACCTGTTTTTGAC
578	MMLV L280A Btm SDM	GGTCTCCTTACGCGCTTCAGTCGCCCCAACGTTGAC CTTCTTTTCAGCA
579	MMLV L280R Btm SDM	GGTCTCCTTACGCGCTTCAGTACGCCAACGTTGAC CTTCTTTTCAGCA
580	MMLV L280E Btm SDM	GGTCTCCTTACGCGCTTCAGTCTCCCAACGTTGAC CTTCTTTTCAGCA
581	MMLV L357A Btm SDM	GGCTTGGTTAAATCCGGAAGACCCGCCGCGGGTGC GGTCAACAAA
582	MMLV L357R Btm SDM	GGCTTGGTTAAATCCGGAAGACCACGCGCGGGTGC GGTCAACAAA
583	MMLV L357E Btm SDM	GGCTTGGTTAAATCCGGAAGACCCTCCGCGGGTGC GGTCAACAAA
584	MMLV T328A Btm SDM	CCAGTTGAAAAGCGTCCCTGTTTTCGCTAAGGGGT ACAGGGGTGCAG
585	MMLV T328R Btm SDM	CCAGTTGAAAAGCGTCCCTGTTTTACGTAAGGGGT ACAGGGGTGCAG
586	MMLV T328E Btm SDM	CCAGTTGAAAAGCGTCCCTGTTTTCTCTAAGGGGT ACAGGGGTGCAG
587	MMLV G331A Btm SDM	GGCCCCCAGTTGAAAAGCGTCGCTGTTTTGTGTTAA GGGGTACAGGGG
588	MMLV G331R Btm SDM	GGCCCCCAGTTGAAAAGCGTACGTGTTTTGTGTTAA GGGGTACAGGGG
589	MMLV G331E Btm SDM	GGCCCCCAGTTGAAAAGCGTCTCTGTTTTGTGTTAA GGGGTACAGGGG

590	MMLV T332A Btm SDM	GTCTGGCCCCCAGTTGAAAAGCGCCCCTGTTTTTG TTAAGGGGTACAG
591	MMLV T332R Btm SDM	GTCTGGCCCCCAGTTGAAAAGACGCCCTGTTTTTG TTAAGGGGTACAG
592	MMLV T332E Btm SDM	GTCTGGCCCCCAGTTGAAAAGCTCCCCTGTTTTTG TTAAGGGGTACAG
593	MMLV N335A Btm SDM	TTTGCTGGTCTGGCCCCCAGCGAAAAGCGTCCCT GTTTTTGTTAAGG
594	MMLV N335R Btm SDM	TTTGCTGGTCTGGCCCCCAACGAAAAGCGTCCCT GTTTTTGTTAAGG
595	MMLV N335E Btm SDM	TTTGCTGGTCTGGCCCCCACTCGAAAAGCGTCCCT GTTTTTGTTAAGG
596	MMLV E367A Btm SDM	ATATCCCTGTTTTTCATCAACGAACAGCGCAAAGG GCTTGGTAAATCCGGAAG
597	MMLV E367R Btm SDM	ATATCCCTGTTTTTCATCAACGAACAGACGAAAGG GCTTGGTAAATCCGGAAG
598	MMLV E367D Btm SDM	ATATCCCTGTTTTTCATCAACGAACAGATCAAAGG GCTTGGTAAATCCGGAAG
599	MMLV F369A Btm SDM	CTTTTGCATATCCCTGTTTTTCATCAACCGCCAGC TCAAAGGGCTTGGTTAAATC
600	MMLV F369R Btm SDM	CTTTTGCATATCCCTGTTTTTCATCAACACGCAGC TCAAAGGGCTTGGTTAAATC
601	MMLV F369E Btm SDM	CTTTTGCATATCCCTGTTTTTCATCAACCTCCAGC TCAAAGGGCTTGGTTAAATC
602	MMLV R389A Btm SDM	TTACTCAAGTAAGCAACAGGGCGCGCCACGGGCC TAACTTTTGGG
603	MMLV R389K Btm SDM	TTACTCAAGTAAGCAACAGGGCGTTTCCACGGGCC TAACTTTTGGG
604	MMLV R389E Btm SDM	TTACTCAAGTAAGCAACAGGGCGCTCCCACGGGCC TAACTTTTGGG
605	MMLV V433A Btm SDM	TCTACAGCATGTGGAGCCAAGATCGCTAAGGGTTG ACCCATCGTCAACT
606	MMLV V433R Btm SDM	TCTACAGCATGTGGAGCCAAGATACGTAAGGGTTG ACCCATCGTCAACT
607	MMLV V433E Btm SDM	TCTACAGCATGTGGAGCCAAGATCTCTAAGGGTTG ACCCATCGTCAACT
608	MMLV V476A Btm SDM	GAAGCAAAGTAGCTGGATTCAAAGCCGCAACTGGT CCAAATTGTACACGATCC
609	MMLV V476R Btm SDM	GAAGCAAAGTAGCTGGATTCAAAGCACGAACTGGT CCAAATTGTACACGATCC
610	MMLV V476E Btm SDM	GAAGCAAAGTAGCTGGATTCAAAGCCTCAACTGGT CCAAATTGTACACGATCC
611	MMLV I593A Btm	GCGGCGGTAAATTTTCGCCATGCGCATGCGCTGTTG

	SDM	CAAAGCATAACG
612	MMLV I593R Btm SDM	GCGGCGGTAAATTTGCCATGACGATGCGCTGTTG CAAAGCATAACG
613	MMLV I593E Btm SDM	GCGGCGGTAAATTTGCCATGCTCATGCGCTGTTG CAAAGCATAACG
614	MMLV E596A Btm SDM	GACCACGGCGGCGGTAAATCGCGCCATGGATATGC GCTGTTGC
615	MMLV E596R Btm SDM	GACCACGGCGGCGGTAAATACGGCCATGGATATGC GCTGTTGC
616	MMLV E596D Btm SDM	GACCACGGCGGCGGTAAATATCGCCATGGATATGC GCTGTTGC
617	MMLV I597A Btm SDM	CAGACCACGGCGGCGGTACGCTTCGCCATGGATAT GCGCTGTTG
618	MMLV I597R Btm SDM	CAGACCACGGCGGCGGTAACGTTTCGCCATGGATAT GCGCTGTTG
619	MMLV I597E Btm SDM	CAGACCACGGCGGCGGTACTCTTCGCCATGGATAT GCGCTGTTG
620	MMLV R650A Btm SDM	GGGCAGCTTGGTCCGCCATCGCGTTTCCACGAGCC TCCGCT
621	MMLV R650K Btm SDM	GGGCAGCTTGGTCCGCCATTTTGTTCACGAGCC TCCGCT
622	MMLV R650E Btm SDM	GGGCAGCTTGGTCCGCCATCTCGTTTCCACGAGCC TCCGCT
623	MMLV Q654A Btm SDM	GCCGCCTTACGGGCAGCCGCGTCCGCCATACGGTT TCCAC
624	MMLV Q654R Btm SDM	GCCGCCTTACGGGCAGCACGGTCCGCCATACGGTT TCCAC
625	MMLV Q654E Btm SDM	GCCGCCTTACGGGCAGCCTCGTCCGCCATACGGTT TCCAC
626	MMLV R657A Btm SDM	GTCTCTGTGATCGCCGCCTTCGCGGCAGCTTGGTC CGCCATA
627	MMLV R657K Btm SDM	GTCTCTGTGATCGCCGCCTTTTTGGCAGCTTGGTC CGCCATA
628	MMLV R657E Btm SDM	GTCTCTGTGATCGCCGCCTTCTCGGCAGCTTGGTC CGCCATA
629	MMLV L280R Top SDM V2	ATTTGCTGAAAGAAGGTCAACGTTGGCGTACTGAT GCGCGTAAGGAGACC
630	MMLV L280R Btm SDM V2	GGTCTCCTTACGCGCATCAGTACGCCAACGTTGAC CTTCTTTCAGCAAAT
631	MMLV L82R Top SDM V2	GGGATTAAGCCACATATTCGTCGCTTGCGTGACCA GGGGATCTTGGTCCC
632	MMLV L82R Btm SDM V2	GGGACCAAGATCCCCTGGTCACGCAAGCGACGAAT ATGTGGCTTAATCCC

Example 2: Preparation of Reverse Transcriptase Mutants for Screening Increased Activity and Thermostability*a. Overexpression of MMLV RTase and mutant variants*

A test induction was used to determine optimum growing conditions. A colony, with the appropriate strain, was used to inoculate Terrific Broth (TB) media (50 mL) with kanamycin (0.05 mg/mL) and grown at 37°C until an OD of approximately 0.9 was reached. The 50 mL culture was divided in half to accommodate two induction temperatures. IPTG (1M; 12.5 µL) was used to induce protein expression, followed by growth at two induction temperatures for 21 hours. Aliquots (normalized to an OD of 1.25) were taken at 3 and 21 hours, cells were harvested at 13,000 x g for one minute, and harvested cells were stored at -20°C. Cells were resuspended in 1x SDS-PAGE running buffer (270 µL) and 5x SDS-PAGE loading dye (70 µL). Samples were boiled for 5 minutes, sonicated, and loaded (15 µL) onto a 4-20% Mini-PROTEAN® TGX Stain-Free™ Protein Gel (Bio Rad, Cat #4568094). SDS-PAGE images are shown in Figure 2.

b. Expression and purification of MMLV RTase and mutant variants

A colony with the appropriate strain was used to inoculate TB media (1 mL, in a 96-well deep well plate) with kanamycin (0.05 mg/mL) and grown at 37°C until an OD of approximately 0.9 was achieved followed by cooling of the plate on ice for 5 minutes. Protein expression was induced by the addition of 100 mM IPTG (5 µL), followed by growth at 18°C for 21 hours. Cells were harvested by spinning samples at 4,700 x g for 10 minutes.

Cell pellets were re-suspended in a lysis buffer (50 mM NaPO₄, pH 7.8, 5% glycerol, 300 mM NaCl, and 10 mM imidazole) and lysed by the addition of 1x BugBuster® (Millipore Sigma, Cat #70921) and incubation on an end-over-end mixer for 15 minutes at room temperature. Cell debris was removed by centrifuging the lysate at 16,000 x g for 20 minutes at 4°C.

Cleared lysates were applied to a HisPur™ Ni-NTA spin plate (ThermoFisher, Cat #88230). Resin was equilibrated with Screening His-Bind buffer (50 mM NaPO₄, pH 7.8, 5% glycerol, 300 mM NaCl, and 10 mM imidazole) and samples loaded. Samples were washed three times with Screening His-Wash buffer (50 mM NaPO₄, pH 7.8, 5% glycerol, 300 mM NaCl, and 25 mM imidazole) and eluted using Screening His-Elution buffer (50 mM NaPO₄, pH 7.8, 5% glycerol, 300 mM NaCl, and 250 mM imidazole). Purified proteins were normalized to a set concentration (100 nM) for testing purposes.

Example 3: Evaluation of Reverse Transcriptase Mutants*a. Evaluation of ability of RTase mutants to synthesize DNA*

The ability of mutant RTase to synthesize cDNA from purified total RNA (DNase-d, isolated from HeLa cells) was compared to an MMLV RTase base construct (RNase H minus construct). Mutant MMLV RTases were tested in two formats: (1) standard two-step cDNA synthesis with gene specific primers, followed by qPCR, and (2) one-step addition of the RTase in Integrated DNA Technologies PrimeTime® Gene Expression Master Mix (GEM).

b. Standard two-step procedure

RTases (2 μ L, 100 nM) were added to a reaction mixture containing RNA (50 ng), dNTPs (100 μ M), gene specific primer set (500 nM; *see* Table 2), first strand synthesis buffer (1x, 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, 10 mM DTT), and SuperaseIN (0.17 U/ μ L) in a 50 μ L volume. The reaction was allowed to proceed at 50°C for 15 minutes, followed by incubation at 80°C for 10 minutes.

cDNA synthesized by RTase mutants was quantified by qPCR amplification using an assay that identified the SFRS9 gene in human cells. The assay master mix composition included GEM (1x), ROX (50 nM), SFRS9 primer set (500 nM; *see* Table 2), and SFRS9 probe (250 nM; *see* Table 2). Assay master mix and synthesized cDNA were mixed at a 4:1 ratio for a final volume of 20 μ L. The reaction was run on qPCR (QuantStudio) for 40 cycles under the following cycle conditions: 95°C hold for 3 minutes, 95°C for 15 seconds, and 60°C for one minute.

Table 2. Sequences of primers and probes used for qPCR assays.

SEQ ID NO:	Primer Name	Primer Sequence (5'–3')
633	Hs SFRS9 Forward Primer	GTCGAGTATCTCAGAAAAGAAGACA
634	Hs SFRS9 Reverse Primer	CTCGGATGTAGGAAGTTTCACC
635	Hs SFRS9 Probe - SUN	/5SUN/ATGCCCTGC/ZEN/GTAAACTGGATGACA/3IABkFQ/

c. One-step procedure in GEM

RTases (1 μ L, 100 nM) were added to a reaction mixture containing RNA (10 ng), GEM (1x), ROX (50 nM), SFRS9 primer set (500 nM; *see* Table 2), and SFRS9 probe (250 nM; *see* Table 2) in a final volume of 20 μ L. The reaction was run on a qPCR machine

(QuantStudio) for 40 cycles using the following cycle conditions: 60°C hold for 15 minutes, 95°C hold for 3 minutes, 95°C for 15 seconds, and 60°C for one minute.

d. MMLV RTase base construct and single mutant variants

As described in Example 1, MMLV RTase single mutant variants were prepared by introducing selected mutations into the MMLV RTase base construct by site-directed mutagenesis, using standard PCR conditions and primers. The sequences of the MMLV RTase base construct and single mutant variants are shown in Table 3. One of skill in the art will understand that the MMLV RTase amino acid sequence set forth in SEQ ID NO: 637 is a truncated form of the full-length amino acid sequence of wild-type, or naturally occurring, MMLV RTase. In addition, a person having ordinary skill in the art will understand that a methionine residue is required to recombinantly produce the MMLV RTase base construct and mutants of the disclosure, and as such, that the MMLV RTase sequences disclosed herein (*see, e.g.*, Tables 3, 8 and 9) include a methionine residue at the N-terminal end of the amino acid sequence. However, with respect to the present disclosure and for the purpose of identifying and numbering residues in the MMLV RTase amino acid sequence where mutations have been introduced, this methionine residue is considered to be amino acid residue 0 (*i.e.*, is not counted) and the second amino acid residue (*e.g.*, threonine in the MMLV RTase base construct set forth in SEQ ID NO: 637) is considered to be amino acid residue 1.

Table 3. Sequences of MMLV RTase base construct and single mutant MMLV RTase constructs.

SEQ ID NO:	Construct	Construct Sequence (DNA: 5'–3' or AA)
636	MMLV RTase	ATGACTTTAAATATTGAGGATGAGCATCGTTTA CATGAGACATCAAAAAGAACCCGACGTGAGCTTA GGGTCAACGTGGCTTTCTGACTTCCCCAGGCG TGGGCGGAGACTGGCGGAATGGGGTTAGCTGTC CGCCAAGCACCGTTGATCATCCCGTTAAAGGCA ACGTCTACACCTGTCTCTATCAAACAGTACCCC ATGAGTCAAGAGGCCCGCCTGGGGATTAAGCCA CATATTCAGCGCTTGCTGGACCAGGGGATCTTG GTCCCATGTCAATCTCCGTGGAACACCCCCCTT CTGCCCCGTGAAAAAGCCAGGTACAAACGATTAT CGTCCAGTTCAAGATCTTCGCGAGGTCAACAAA CGCGTAGAAGACATCCATCCGACTGTACCTAAT CCTTATAATCTGTTATCAGGCCTGCCCCATCG CACCAATGGTATACAGTATTAGACTTGAAAGAC GCGTTCTTTTGCTGCGTCTGCACCCAACGTCT CAGCCGCTGTTTGCGTTCGAATGGCGTGATCCT

		GAAATGGGAATTTTCGGGTCAGTTAACCTGGACT CGTCTGCCCCAGGGCTTTAAAAACAGCCCCACA TTGTTTCGATGAAGCACTTCACCGTGACTTAGCA GACTTCCGTATCCAACACCCAGACTTAATTCTG TTACAGTATGTTGACGACCTTTTGTGGCGGCA ACGTCTGAACTTGACTGTCAGCAAGGCACACGC GCGTTATTACAAACGTTAGGTAACTTAGGATAT CGTGCGTCCGCGAAAAAGGCGCAAATTTGTCAA AAACAGGTAAAGTACCTTGGGTATTTGCTGAAA GAAGGTCAACGTTGGCTGACTGAAGCGCGTAAG GAGACCGTAATGGGGCAGCCTACGCCTAAGACG CCACGCCAGTTGCGTGAATTTTTGGGCACAGCG GGATTCTGTCGTTTATGGATTCTTGGGTTTCGCT GAAATGGCTGCACCCCTGTACCCCTTAACAAAA ACAGGGACGCTTTTCAACTGGGGGCCAGACCAG CAAAAGGCGTATCAGGAGATCAAACAAGCTTTG TTGACCGCACCCGCGTTGGGTCTTCCGGATTTA ACCAAGCCCTTTGAGCTGTTTCGTTGATGAAAA CAGGGATATGCAAAAGGAGTATTAACCCAAAAG TTAGGCCCCGTGGCGTCGCCCTGTTGCTTACTTG AGTAAAAAATTGGATCCTGTCGCAGCAGGATGG CCACCGTGCTTGCGTATGGTCGCGGCAATTGCC GTTTTGACAAAGGATGCAGGTAAGTTGACGATG GGTCAACCCTTAGTAATCTTGGCTCCACATGCT GTAGAAGCGTTAGTAAAGCAGCCCCCAGACCGC TGGCTTTCTAATGCGCGCATGACCCACTATCAG GCGCTTCTGCTTGATACGGATCGTGTAACAATTT GGACCAGTTGTAGCTTTGAATCCAGCTACTTTG CTTCCCTTCCAGAAGAAGGACTTCAGCACAAAT TGTTTAGATATTCTGGCCGAGGCACATGGGACG CGCCCTGATTTGACGGATCAGCCACTGCCTGAT GCCGACCATACATGGTATACTGGCGGCAGTAGT CTTCTTCAAGAGGGGCAACGCAAGGCGGGAGCA GCCGTCACTACGGAGACCGAAGTTATCTGGGCC AAAGCGTTACCCGCGGGAACATCCGCGCAACGT GCACAGTTAATCGCTCTGACACAGGCCCTGAAG ATGGCAGAGGGCAAAAAGTTGAATGTCTACACC AACTCACGTTATGCTTTTGCAACAGCGCATATC CATGGCGAAATTTACCGCCGCCGTGGTCTGCTG ACTAGTGAGGGTAAGGAAATTAAAAATAAAGAT GAGATTCTTGCGTTGTTAAAAGCTTTATTCTTA CCAAAACGCCTTTCGATCATTATTGCCCGGGG CATCAAAAGGGTCACTCAGCGGAGGCTCGTGGA AACCGTATGGCGGACCAAGCTGCCCCGTAAGGCG GCGATCACAGAGACCCCGGATACATCAACGCTG TTGATCGAAAACAGCTCTCCCTACACTAGCGAG CATTTTTAA
637	MMLV RTase	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSIIKQYP MSQEARLGIKPHIQRLDQGILVPCQSPWNTP

		LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCCQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
638	MMLV RTase with I61R mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVS RKQYP MSQEARLG I KPHIQRLDQ GILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCCQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
639	MMLV RTase with Q68R mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVS I KQYP MSREARLG I KPHIQRLDQ GILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCCQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL

		TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
640	MMLV RTase with Q79R mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPKATSTPVS IKQYP MSQEARLG I KPHIRRLDQ GILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR IQHPDLILLQYVDDL LLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLTEARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
641	MMLV RTase with L99R mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPKATSTPVS IKQYP MSQEARLG I KPHIRRLDQ GILVPCQSPWNTPR LPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR IQHPDLILLQYVDDL LLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLTEARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL

		PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
642	MMLV RTase with E282D mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVS IKQYP MSQEARLG I KPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPT LFDEALHRDLADFR IQHPDLILLQYVDDLL LAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLTDARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTN SRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
643	MMLV RTase with R298A mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVS IKQYP MSQEARLG I KPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPT LFDEALHRDLADFR IQHPDLILLQYVDDLL LAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLTEARKETVMGQPTPKT PAQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTN SRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF

e. Experimental results

The two-step and one-step reactions for MMLV RTase base construct and MMLV RTase single mutant variants were analyzed and reported by copy number output based on a

standard curve (*see* Tables 4 and 5). Six single mutant MMLV RTase variants were found to exhibit an increase in the overall activity and thermostability as compared to the MMLV RTase base construct. The six single mutant MMLV RTase variants were as follows: I61R, Q68R, Q79R, L99R, E282D, and R298A.

Table 4. Two-step cDNA synthesis by MMLV RT single mutants. Data was generated via qPCR human normalizer assay and translated by copy number.

MMLV RT Variant	Quantity Mean	Quantity Standard Deviation
MMLV-II	21,046.784	954.827
MMLV-II A283V	280.423	50.910
MMLV-II A283R	10,390.819	340.236
MMLV-II A283E	7,378.705	122.716
MMLV-II E123A	15,059.791	556.095
MMLV-II E123R	19,043.292	415.522
MMLV-II E123D	3,619.959	243.766
MMLV-II E282A	19,939.551	1,645.246
MMLV-II E282R	15,588.940	546.467
MMLV-II E282D	24,282.327	2,259.264
MMLV-II I61A	648.252	45.640
MMLV-II I61R	26,280.811	549.417
MMLV-II I61E	10,966.741	469.747
MMLV-II K102A	98.438	12.778
MMLV-II K102R	780.114	90.331
MMLV-II K102E	1,674.854	157.485
MMLV-II K103A	359.984	67.322
MMLV-II K103R	206.765	20.758
MMLV-II K103E	200.883	16.719
MMLV-II K120A	217.787	72.696
MMLV-II K120R	3,619.338	100.478
MMLV-II K120E	2,230.375	210.050
MMLV-II K193A	2,736.271	162.383
MMLV-II K193R	11,496.935	193.681
MMLV-II K193E	325.109	50.932
MMLV-II K295A	8,101.927	348.373
MMLV-II K295R	6,879.112	131.993
MMLV-II K295E	9,673.612	351.106
MMLV-II K329A	3,199.167	212.003
MMLV-II K329R	10,387.670	330.429
MMLV-II K329E	18,306.813	1,167.600
MMLV-II K53A	474.465	62.390
MMLV-II K53R	369.020	49.436
MMLV-II K53E	5,308.165	104.585
MMLV-II K62A	2,102.396	64.197
MMLV-II K62R	4,920.330	251.414
MMLV-II K62E	71.723	11.419
MMLV-II K75A	76.659	24.657

MMLV-II K75R	2,842.314	77.212
MMLV-II K75E	1,697.887	158.946
MMLV-II L99A	1,576.246	213.455
MMLV-II L99R	37,070.048	1,531.910
MMLV-II L99E	195.448	22.530
MMLV-II N107A	3,354.325	176.385
MMLV-II N107R	41.532	24.527
MMLV-II N107E	8,523.285	353.411
MMLV-II Q291A	14,093.444	576.318
MMLV-II Q291R	15,736.443	566.630
MMLV-II Q291E	1,480.309	93.187
MMLV-II Q68A	n.d.	n.d.
MMLV-II Q68R	20,158.035	722.022
MMLV-II Q68E	2,263.714	150.236
MMLV-II Q79A	2,317.484	43.518
MMLV-II Q79R	37,480.443	1,268.309
MMLV-II Q79E	489.184	39.449
MMLV-II R110A	1,815.710	7.917
MMLV-II R110K	502.172	38.619
MMLV-II R110E	383.331	38.162
MMLV-II R298A	44,477.013	3,036.502
MMLV-II R298K	14,925.202	186.581
MMLV-II R298E	1,150.932	56.107
MMLV-II R301A	2,745.075	82.646
MMLV-II R301K	12,813.899	568.898
MMLV-II R301E	1,583.826	198.913
MMLV-II T106A	16,641.642	179.631
MMLV-II T106R	2,248.217	71.295
MMLV-II T106E	10,302.113	250.531
MMLV-II T128V	7,034.032	351.446
MMLV-II T128R	3,465.069	143.456
MMLV-II T128E	10,709.019	110.124
MMLV-II T293A	4,612.880	167.335
MMLV-II T293R	13,753.879	319.851
MMLV-II T293E	12,893.457	223.100
MMLV-II T296A	2,192.531	76.071
MMLV-II T296R	893.449	51.913
MMLV-II T296E	473.936	102.414
MMLV-II T55A	5,774.471	223.173
MMLV-II T55R	3,284.089	314.651
MMLV-II T55E	6,143.058	429.507
MMLV-II T57A	6,129.791	285.070
MMLV-II T57R	888.244	11.952
MMLV-II T57E	1,487.448	71.681
MMLV-II V101A	552.130	98.391
MMLV-II V101R	4,754.017	107.434
MMLV-II V101E	1,388.699	87.091
MMLV-II V112A	2,085.594	72.265

MMLV-II V112R	377.194	41.722
MMLV-II V112E	210.825	17.715
MMLV-II V59A	628.779	15.216
MMLV-II V59R	6,662.173	210.234
MMLV-II V59E	3,249.465	79.848
MMLV-II Y109A	101.656	6.717
MMLV-II Y109R	349.373	27.171
MMLV-II Y109E	1,029.589	45.189
MMLV-IV	71,572.714	4,656.679

Table 5. One-step cDNA synthesis by MMLV RT single mutants. Data was generated via qPCR human normalizer assay and data is translated by copy number.

MMLV RT Variant	Quantity Mean	Quantity Standard Deviation
MMLV-II	20,638.973	614.785
MMLV-II A283V	8,802.753	220.902
MMLV-II A283R	14,379.575	337.562
MMLV-II A283E	16,396.614	203.476
MMLV-II E123A	17,975.218	259.986
MMLV-II E123R	20,652.508	515.600
MMLV-II E123D	14,452.672	242.000
MMLV-II E282A	19,017.751	827.419
MMLV-II E282R	17,180.421	204.739
MMLV-II E282D	20,735.271	420.881
MMLV-II I61A	7,450.147	348.788
MMLV-II I61R	25,123.507	2,977.836
MMLV-II I61E	17,441.860	1,662.749
MMLV-II K102A	9,342.754	120.846
MMLV-II K102R	10,563.589	255.139
MMLV-II K102E	13,925.008	307.601
MMLV-II K103A	9,429.555	437.351
MMLV-II K103R	9,009.846	155.888
MMLV-II K103E	7,985.278	189.792
MMLV-II K120A	8,593.433	438.722
MMLV-II K120R	12,558.793	407.946
MMLV-II K120E	12,268.574	303.495
MMLV-II K193A	12,977.263	537.992
MMLV-II K193R	13,446.766	2,337.906
MMLV-II K193E	8,536.558	182.514
MMLV-II K295A	13,506.491	1,613.467
MMLV-II K295R	13,944.407	1,839.608
MMLV-II K295E	15,021.823	650.111
MMLV-II K329A	13,284.541	246.298
MMLV-II K329R	15,935.899	970.971
MMLV-II K329E	20,628.859	884.254
MMLV-II K53A	10,868.676	161.435
MMLV-II K53R	9,908.252	632.663
MMLV-II K53E	20,666.775	518.895

MMLV-II K62A	9,454.043	732.242
MMLV-II K62R	14,532.171	63.450
MMLV-II K62E	8,341.361	436.076
MMLV-II K75A	9,084.502	113.100
MMLV-II K75R	13,106.462	331.663
MMLV-II K75E	11,191.849	565.160
MMLV-II L99A	12,876.076	49.507
MMLV-II L99R	27,167.197	142.371
MMLV-II L99E	6,534.199	2,730.598
MMLV-II N107A	13,563.421	349.378
MMLV-II N107R	8,654.167	497.167
MMLV-II N107E	16,675.075	172.596
MMLV-II Q291A	20,957.729	150.006
MMLV-II Q291R	17,980.723	346.436
MMLV-II Q291E	11,025.722	407.116
MMLV-II Q68A	n.d.	n.d.
MMLV-II Q68R	24,925.791	937.265
MMLV-II Q68E	12,844.484	165.039
MMLV-II Q79A	12,038.975	482.596
MMLV-II Q79R	28,458.521	296.595
MMLV-II Q79E	10,358.863	309.043
MMLV-II R110A	11,517.764	562.094
MMLV-II R110K	8,112.167	76.742
MMLV-II R110E	8,809.423	290.785
MMLV-II R298A	27,817.905	172.690
MMLV-II R298K	18,222.660	825.743
MMLV-II R298E	10,783.790	783.279
MMLV-II R301A	11,344.854	63.499
MMLV-II R301K	17,584.850	445.587
MMLV-II R301E	10,146.906	1,879.902
MMLV-II T106A	17,717.520	215.965
MMLV-II T106R	11,680.187	148.213
MMLV-II T106E	21,203.557	366.469
MMLV-II T128V	14,384.970	355.754
MMLV-II T128R	12,938.223	464.841
MMLV-II T128E	14,781.394	1,930.931
MMLV-II T293A	15,658.189	347.640
MMLV-II T293R	19,976.165	253.604
MMLV-II T293E	17,580.335	404.397
MMLV-II T296A	10,312.142	159.775
MMLV-II T296R	8,482.071	92.806
MMLV-II T296E	7,687.972	112.884
MMLV-II T55A	18,073.262	618.174
MMLV-II T55R	11,546.179	138.906
MMLV-II T55E	12,299.658	815.911
MMLV-II T57A	14,700.042	2,916.521
MMLV-II T57R	11,195.901	145.433
MMLV-II T57E	11,958.503	605.445

MMLV-II V101A	10,697.751	269.696
MMLV-II V101R	8,934.765	53.924
MMLV-II V101E	11,295.874	296.506
MMLV-II V112A	12,854.738	356.724
MMLV-II V112R	6,331.802	303.453
MMLV-II V112E	7,643.184	448.446
MMLV-II V59A	9,520.143	339.954
MMLV-II V59R	18,523.053	499.377
MMLV-II V59E	16,029.631	137.454
MMLV-II Y109A	8,421.361	185.196
MMLV-II Y109R	8,581.961	129.732
MMLV-II Y109E	10,216.473	416.388
MMLV-IV	65,726.159	1,811.314

Example 4: Extension of Reverse Transcriptase Single Mutants

The amino acid positions that enclosed the MMLV RTase single mutants identified in Example 3 were further evaluated to include all possible amino acid substitutions at that position. The single mutants were cloned, overexpressed, and purified as described in Examples 1 and 2, and evaluated as described in Example 3. The two-step and one-step reactions for MMLV RTase base construct and MMLV RTase double mutant variants were analyzed and reported by copy number output based on a standard curve (*see* Tables 6 and 7). Ten single mutant MMLV RTase variants (*see* Table 8) were found to exhibit an increase in the overall activity and thermostability as compared to the MMLV RTase base construct. The ten single mutant MMLV RTase variants were as follows: I61K, I61M, Q68I, Q68K, Q79H, Q79I, L99K, L99N, E282M and E282W.

Table 6. Two-step cDNA synthesis by MMLV RT single mutants. Data was generated via qPCR human normalizer assay and translated by copy number.

MMLV RT Variant	Quantity Mean	Quantity Standard Deviation
MMLV-II	1,484.121	125.278
MMLV-II E282C	749.332	37.947
MMLV-II E282F	968.042	28.112
MMLV-II E282G	841.839	30.618
MMLV-II E282H	936.562	64.904
MMLV-II E282I	1,418.551	8.682
MMLV-II E282K	2,399.973	50.862
MMLV-II E282L	1,778.903	134.133
MMLV-II E282M	2,115.328	125.477
MMLV-II E282N	1,175.130	79.221
MMLV-II E282P	1,529.331	61.525
MMLV-II E282Q	1,856.418	24.118
MMLV-II E282S	673.670	44.770

MMLV-II E282T	994.318	24.066
MMLV-II E282V	748.877	29.053
MMLV-II E282W	2,469.404	141.080
MMLV-II E282Y	1,360.706	338.309
MMLV-II I61C	283.240	11.244
MMLV-II I61D	349.008	10.979
MMLV-II I61F	784.163	22.643
MMLV-II I61G	395.348	21.967
MMLV-II I61H	736.015	30.271
MMLV-II I61K	4,479.606	62.627
MMLV-II I61L	1,106.547	38.553
MMLV-II I61M	4,198.088	93.025
MMLV-II I61N	709.752	29.312
MMLV-II I61P	32.935	16.814
MMLV-II I61Q	1,311.695	145.810
MMLV-II I61S	797.783	50.626
MMLV-II I61T	628.173	33.371
MMLV-II I61V	1,439.915	27.490
MMLV-II I61W	442.039	29.310
MMLV-II I61Y	534.249	26.831
MMLV-II L99C	3,109.142	80.016
MMLV-II L99D	83.653	3.432
MMLV-II L99F	2,811.513	79.584
MMLV-II L99G	908.041	16.157
MMLV-II L99H	4,881.196	390.497
MMLV-II L99I	910.072	71.671
MMLV-II L99K	6,410.818	127.262
MMLV-II L99M	976.548	65.154
MMLV-II L99N	4,974.458	162.464
MMLV-II L99P	6.416	1.820
MMLV-II L99Q	3,908.473	337.167
MMLV-II L99S	3,793.955	86.959
MMLV-II L99T	4,189.211	27.640
MMLV-II L99V	964.081	48.105
MMLV-II L99W	1,614.660	40.442
MMLV-II L99Y	2,123.406	181.945
MMLV-II Q68A	1,184.702	7.676
MMLV-II Q68C	2,038.167	36.463
MMLV-II Q68D	1,613.880	77.796
MMLV-II Q68F	1,805.647	62.456
MMLV-II Q68G	2,262.873	69.688
MMLV-II Q68H	106.421	9.860
MMLV-II Q68I	2,675.446	73.874
MMLV-II Q68K	1,042.979	70.081
MMLV-II Q68L	1,070.742	57.215
MMLV-II Q68M	1,342.806	58.349
MMLV-II Q68N	1,993.946	65.808
MMLV-II Q68P	2,025.753	25.540

MMLV-II Q68S	1,895.984	26.959
MMLV-II Q68T	431.442	22.751
MMLV-II Q68V	1,534.710	110.794
MMLV-II Q68W	1,790.706	124.583
MMLV-II Q79C	2,477.812	107.510
MMLV-II Q79D	627.902	11.073
MMLV-II Q79F	1,786.571	126.904
MMLV-II Q79G	2,702.985	83.998
MMLV-II Q79H	2,851.710	57.501
MMLV-II Q79I	2,967.710	57.440
MMLV-II Q79K	1,346.751	64.513
MMLV-II Q79L	2,214.615	67.622
MMLV-II Q79M	1,847.181	31.384
MMLV-II Q79N	1,365.563	54.775
MMLV-II Q79P	674.074	42.100
MMLV-II Q79S	2,199.353	52.958
MMLV-II Q79T	1,523.163	77.025
MMLV-II Q79V	1,704.661	77.643
MMLV-II Q79W	2,186.489	31.470
MMLV-II Q79Y	2,326.023	123.508
MMLV-II R298C	79.970	9.815
MMLV-II R298D	0.000	0.000
MMLV-II R298F	84.760	9.362
MMLV-II R298G	357.027	15.726
MMLV-II R298H	269.257	20.814
MMLV-II R298I	130.983	5.364
MMLV-II R298L	199.612	5.843
MMLV-II R298M	172.013	18.710
MMLV-II R298N	199.678	2.660
MMLV-II R298P	122.098	5.900
MMLV-II R298Q	118.092	40.694
MMLV-II R298S	406.112	7.695
MMLV-II R298T	618.616	20.023
MMLV-II R298V	136.498	13.297
MMLV-II R298W	68.096	7.016
MMLV-II R298Y	162.713	7.854
MMLV-IV	6,830.294	376.878

Table 7. One-step cDNA synthesis by MMLV RT single mutants. Data was generated via qPCR human normalizer assay and data is translated by copy number.

MMLV RT Variant	Quantity Mean	Quantity Standard Deviation
MMLV-II	408.018	8.693
MMLV-II E282C	175.083	7.005
MMLV-II E282F	1,043.025	16.137
MMLV-II E282G	635.037	13.293

MMLV-II E282H	656.956	10.018
MMLV-II E282I	1,033.125	44.996
MMLV-II E282K	751.309	17.611
MMLV-II E282L	1,072.350	80.365
MMLV-II E282M	1,318.072	51.735
MMLV-II E282N	539.305	10.767
MMLV-II E282P	725.869	92.685
MMLV-II E282Q	626.674	12.129
MMLV-II E282S	354.956	34.850
MMLV-II E282T	485.477	45.783
MMLV-II E282V	594.047	27.898
MMLV-II E282W	913.290	61.145
MMLV-II E282Y	759.920	34.784
MMLV-II I61C	219.438	18.403
MMLV-II I61D	347.020	13.303
MMLV-II I61F	428.623	25.316
MMLV-II I61G	389.503	21.764
MMLV-II I61H	514.330	18.416
MMLV-II I61K	2,343.894	67.214
MMLV-II I61L	621.572	14.892
MMLV-II I61M	2,536.807	150.371
MMLV-II I61N	538.519	20.736
MMLV-II I61P	61.683	18.802
MMLV-II I61Q	701.471	32.487
MMLV-II I61S	611.977	30.430
MMLV-II I61T	534.254	31.643
MMLV-II I61V	881.608	20.662
MMLV-II I61W	428.440	17.964
MMLV-II I61Y	347.930	4.412
MMLV-II L99C	2,390.104	35.867
MMLV-II L99D	185.044	6.975
MMLV-II L99F	1,577.767	7.757
MMLV-II L99G	987.225	9.718
MMLV-II L99H	3,886.372	111.670
MMLV-II L99I	613.648	46.303
MMLV-II L99K	7,597.650	321.753
MMLV-II L99M	934.817	52.006
MMLV-II L99N	4,689.222	160.641
MMLV-II L99P	18.537	1.131
MMLV-II L99Q	2,394.744	64.077
MMLV-II L99S	3,293.831	111.802
MMLV-II L99T	3,505.113	101.670
MMLV-II L99V	677.756	49.356
MMLV-II L99W	839.088	50.301
MMLV-II L99Y	1,127.536	19.074
MMLV-II Q68A	827.617	30.689
MMLV-II Q68C	1,110.680	45.944
MMLV-II Q68D	1,045.802	25.488

MMLV-II Q68F	1,210.166	120.899
MMLV-II Q68G	907.279	30.688
MMLV-II Q68H	150.384	6.867
MMLV-II Q68I	1,550.372	76.712
MMLV-II Q68K	1,712.176	47.342
MMLV-II Q68L	651.039	51.426
MMLV-II Q68M	1,395.463	34.805
MMLV-II Q68N	1,241.364	25.780
MMLV-II Q68P	1,249.444	13.709
MMLV-II Q68S	1,125.260	21.324
MMLV-II Q68T	792.901	31.513
MMLV-II Q68V	1,026.654	24.972
MMLV-II Q68W	1,594.175	101.221
MMLV-II Q79C	1,948.151	87.341
MMLV-II Q79D	458.131	10.763
MMLV-II Q79F	1,623.675	50.723
MMLV-II Q79G	1,885.097	20.190
MMLV-II Q79H	2,508.763	149.926
MMLV-II Q79I	2,329.030	76.545
MMLV-II Q79K	1,861.302	24.320
MMLV-II Q79L	1,496.247	30.399
MMLV-II Q79M	1,496.469	38.178
MMLV-II Q79N	995.813	42.279
MMLV-II Q79P	526.914	23.216
MMLV-II Q79S	1,685.124	42.694
MMLV-II Q79T	966.505	8.377
MMLV-II Q79V	1,218.191	21.512
MMLV-II Q79W	1,962.326	37.135
MMLV-II Q79Y	2,218.504	56.938
MMLV-II R298C	45.500	1.456
MMLV-II R298D	0.000	0.000
MMLV-II R298F	104.825	5.133
MMLV-II R298G	323.542	14.052
MMLV-II R298H	253.202	47.711
MMLV-II R298I	205.982	8.304
MMLV-II R298L	213.674	15.199
MMLV-II R298M	176.347	12.484
MMLV-II R298N	142.969	39.198
MMLV-II R298P	188.995	3.689
MMLV-II R298Q	95.525	44.292
MMLV-II R298S	307.614	9.962
MMLV-II R298T	487.828	3.480
MMLV-II R298V	255.828	12.902
MMLV-II R298W	37.872	8.482
MMLV-II R298Y	153.333	25.137
MMLV-IV	19,407.721	466.310

Table 8. Sequences of single mutant MMLV RTase variants.

SEQ ID NO:	Construct	Construct Sequence (AA)
644	MMLV RTase with I61K mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS K KQYP MSQEARLG I KPHIQRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREV N KRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR I QHPDL I LLQYVDDLL LAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLTEARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLD TDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLNVYTNSRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLS I IHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
645	MMLV RTase with I61M mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS M KQYP MSQEARLG I KPHIQRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREV N KRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR I QHPDL I LLQYVDDLL LAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLTEARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLD TDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLNVYTNSRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLS I IHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
646	MMLV RTase with Q68I mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS I KQYP MSI EARLG I KPHIQRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREV N KRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR I QHPDL I LLQYVDDLL LAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ

		KQVKYLGYLLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
647	MMLV RTase with Q68K mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVS IKQYP MSKEARLG IKPHIQRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFR IQHPDLILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYLLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
648	MMLV RTase with Q79H mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVS IKQYP MSQEARLG IKPHIHRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFR IQHPDLILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYLLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR

		AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
649	MMLV RTase with Q79I mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSIKQYP MSQEARLGKPHIIRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFRIQHPDLILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGILLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGS LLQEGQRKAGAAVTTEVEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
650	MMLV RTase with L99K mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSIKQYP MSQEARLGKPHIIRLLDQGILVPCQSPWNTPL KPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFRIQHPDLILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGILLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGS LLQEGQRKAGAAVTTEVEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
651	MMLV RTase with L99N mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSIKQYP MSQEARLGKPHIIRLLDQGILVPCQSPWNTPL NPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS

		QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDPQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
652	MMLV RTase with E282M mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSQYP MSQEARLGKPHIQRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLLKEGQRWLTMARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDPQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
653	MMLV RTase with E282W mutation	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSQYP MSQEARLGKPHIQRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLLKEGQRWLTWARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDPQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ

		ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGSS LLQEGQRKAGAAVTTETETEVIAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
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Example 5: Stacking of Reverse Transcriptase Mutants with Enhanced Activity

a. MMLV RTase double mutants

The MMLV RTase single mutants identified in Example 3 were stacked to further improve the ability of MMLV RTase to synthesize cDNA from purified total RNA (DNase-d, isolated from HeLa cells) as compared to the MMLV RTase base construct (RNase H minus construct). Fifteen MMLV RTase double mutant variants (*see* Table 9) were cloned, overexpressed, and purified as described in Examples 1 and 2, and evaluated as described in Example 3. The two-step and one-step reactions for MMLV RTase base construct and MMLV RTase double mutant variants were analyzed and reported by copy number output based on a standard curve (*see* Tables 10 and 11).

Four of the fifteen MMLV RTase double mutant variants were found to exhibit increased overall activity and thermostability as compared to the other MMLV RTase double mutant variants, and almost all of the MMLV RTase double mutant variants exhibited increased overall activity and thermostability as compared to the MMLV RTase base construct. The four MMLV RTase double mutant variants that were found to exhibit the highest overall activity were E282D/L99R, L99R/Q68R, L99R/Q79R, and Q68R/Q79R.

Table 9. Sequences of double mutant MMLV RTase variants.

SEQ ID NO:	Construct	Construct Sequence (AA)
654	MMLV RTase with I61R/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPKATSTPVSARKQYP MSQEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCCQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGILLKEGQRWLTDAKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTKLGPWRRPVAYL SKKLDPVAAAGWPPCLRMVAAIAVLTKDAGKLT MQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ

		ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
655	MMLV RTase with L99R/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS IKQYP MSQEARLG IKPHIQRLLDQGILVPCQSPWNTPR LPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR IQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT DARETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGP DQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRP VAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
656	MMLV RTase with Q68R/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS IKQYP MSREARLG IKPHIQRLLDQGILVPCQSPWNTP L LPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR IQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT DARETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGP DQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRP VAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
657	MMLV RTase with	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA

	Q79R/E282D mutations	WAETGGMGLAVRQAPLI I PLKATSTPVS IKQYP MSQEARLG I KPHIRRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR IQHPDLILLQYVDDLLLAA TSELD CQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT DARKETVMGQTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGT LFNWGP DQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLD TDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTN SRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLS I IHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
658	MMLV RTase with E282D/R298A mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS IKQYP MSQEARLG I KPHIQRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR IQHPDLILLQYVDDLLLAA TSELD CQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT DARKETVMGQTPKT PAQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGT LFNWGP DQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLD TDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTN SRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLS I IHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
659	MMLV RTase with I61R/L99R mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS RKQYP MSQEARLG I KPHIQRLDQGILVPCQSPWNTPL R PVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR IQHPDLILLQYVDDLLLAA TSELD CQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT EARKETVMGQTPKT

		PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
660	MMLV RTase with I61R/Q68R mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSRKQYP MSREARLGKPHIQRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGILLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEH
661	MMLV RTase with I61R/Q79R mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSRKQYP MSQEARLGKPHIRRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGILLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR

		AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
662	MMLV RTase with I61R/R298A mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS RKQYP MSQEARLG I KPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFR IQHPDLILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLTEARKETVMGQPTPKT PAQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRP VAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGSS LLQEGQRKAGAAVTETETEVIAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
663	MMLV RTase with Q68R/L99R mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS I KQYP MSREARLG I KPHIQRLLDQGILVPCQSPWNTPL RPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFR IQHPDLILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLTEARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRP VAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGSS LLQEGQRKAGAAVTETETEVIAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
664	MMLV RTase with Q79R/L99R mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS I KQYP MSQEARLG I KPHIRRLDQGILVPCQSPWNTPL

		RPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCCQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
665	MMLV RTase with L99R/R298A mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSQYP MSQEARLGKPHIQRLDQGILVPCQSPWNTPL RPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCCQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLKEGQRWLTEARKETVMGQPTPKT PAQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
666	MMLV RTase with Q68R/Q79R mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSQYP MSREARLGKPHIRRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCCQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLKEGQRWLTEARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDDQKAYQEIKQALLTAPALGLPDL

		TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
667	MMLV RTase with Q68R/R298A mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPKATSTPVSQKYP MSREARLGKPHIQRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPGGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGILLKEGQRWLTEARKETVMGQPTPKT PAQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
668	MMLV RTase with Q79R/R298A mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPKATSTPVSQKYP MSQEARLGKPHIRRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPGGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGILLKEGQRWLTEARKETVMGQPTPKT PAQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL

		PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
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Table 10. Two-Step cDNA synthesis by MMLV RT double mutants. Data was generated *via* qPCR human normalizer assay and data is translated by copy number.

MMLV RT Variant	Quantity Mean	Quantity Standard Deviation
MMLV-II	1,773.623	5.057
MMLV-II E282D/I61R	4,810.277	143.422
MMLV-II E282D/L99R	7,266.281	50.730
MMLV-II E282D/Q68R	5,186.392	69.563
MMLV-II E282D/Q79R	4,311.403	95.402
MMLV-II E282D/R298A	1,366.524	16.429
MMLV-II I61R/L99R	6,061.812	174.619
MMLV-II I61R/Q68R	5,899.316	39.879
MMLV-II I61R/Q79R	5,257.089	98.378
MMLV-II I61R/R298A	2,661.223	68.948
MMLV-II L99R/Q68R	7,750.519	94.408
MMLV-II L99R/Q79R	7,455.203	124.095
MMLV-II L99R/R298A	5,351.021	179.558
MMLV-II Q68R/Q79R	7,178.681	86.595
MMLV-II Q68R/R298A	4,524.340	84.703
MMLV-II Q79R/R298A	3,739.608	58.621
MMLV-IV	8,258.715	79.458

Table 11. One-Step cDNA synthesis by MMLV RT double mutants. Data was generated *via* qPCR human normalizer assay and data is translated by copy number.

MMLV-RT Variant	Quantity Mean	Quantity Standard Deviation
MMLV-II	859.127	24.795
MMLV-II E282D/I61R	2,948.906	49.177
MMLV-II E282D/L99R	4,814.957	239.110
MMLV-II E282D/Q68R	3,709.046	131.434
MMLV-II E282D/Q79R	3,694.187	98.772
MMLV-II E282D/R298A	794.643	39.913
MMLV-II I61R/L99R	3,443.713	180.210
MMLV-II I61R/Q68R	3,525.138	112.288
MMLV-II I61R/Q79R	3,125.990	120.996
MMLV-II I61R/R298A	2,006.208	83.559
MMLV-II L99R/Q68R	6,755.852	102.788
MMLV-II L99R/Q79R	6,709.502	35.997
MMLV-II L99R/R298A	2,128.451	55.565
MMLV-II Q68R/Q79R	6,343.821	140.779
MMLV-II Q68R/R298A	2,406.470	74.117
MMLV-II Q79R/R298A	2,301.759	22.849
MMLV-IV	15,411.857	333.388

b. Cloning of MMLV RTase triple and more mutants

Following the double mutant variants, MMLV RTase single mutants were stacked further to improve the ability of MMLV RTase to synthesize cDNA from purified total RNA (DNased, isolated from HeLa cells) as compared to the MMLV RTase base construct (RNase H minus construct). Seventeen MMLV RTase triple or more mutant variants (*see* Table 12) were cloned as described in Example 1.

Table 12. Sequences of triple or more mutant MMLV RTase variants.

SEQ ID NO:	Construct	Construct Sequence (AA)
669	MMLV RTase with Q68R/L99R/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVSIKQYP MSREARLG I KPH I QRLLDQGILVPCQSPWNTPL RPVKKPGTNDYRVPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD A FFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR I QHPDL I LLQYVDDLLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT D ARKETVMGQPTPKT PRQLREFLG TAGFCRLWI PGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQE I KQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG P WRRPVAYL SKKLD PVAAGWPPCLRMVAAI AVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTETETEV I WAKALPAGTSAQR AQLIALTQALKMAEGKKNVYTNSRYAFATAHI HGEIYRRRGLLTSEGKE I KNKDE I LALLKALFL PKRLS I I HCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
670	MMLV RTase with Q79R/L99R/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVSIKQYP MSQEARLG I KPH I RRLLDQGILVPCQSPWNTPL RPVKKPGTNDYRVPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD A FFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR I QHPDL I LLQYVDDLLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT D ARKETVMGQPTPKT PRQLREFLG TAGFCRLWI PGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQE I KQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG P WRRPVAYL SKKLD PVAAGWPPCLRMVAAI AVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS

		LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
671	MMLV RTase with Q68R/Q79R/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSIKQYP MSREARLGKPHIRRLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLKEGQRWLT DARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDREVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
672	MMLV RTase with Q68R/Q79R/L99R mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSIKQYP MSREARLGKPHIRRLDQGILVPCQSPWNTPL RPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFRIQHPDLILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLKEGQRWLT EARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDREVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
673	MMLV RTase with Q68R/Q79R/L99R/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSIKQYP MSREARLGKPHIRRLDQGILVPCQSPWNTPL RPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN

		PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYPYLLKEGQRWLT DARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDPQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
674	MMLV RTase with Q68R/Q79R/L99K/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS IKQYP MSREARLG I KPHIRRLDQGILVPCQSPWNTPL KPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYPYLLKEGQRWLT DARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDPQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
675	MMLV RTase with Q68R/Q79R/L99N/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS IKQYP MSREARLG I KPHIRRLDQGILVPCQSPWNTPL NPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFRIQHPDLILLQYVDDLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYPYLLKEGQRWLT DARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDPQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT

		GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
676	MMLV RTase with Q68I/Q79R/L99R/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVS IKQYP MSIEARLG IKPHIRRLDQGILVPCQSPWNTPL RPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVL DLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFR IQHPDLILLQYVDDL LLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT DARETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRP VAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
677	MMLV RTase with Q68K/Q79R/L99R/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVS IKQYP MSKEARLG IKPHIRRLDQGILVPCQSPWNTPL RPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVL DLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFR IQHPDLILLQYVDDL LLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT DARETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRP VAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
678	MMLV RTase with	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA

	Q68R/Q79H/L99R/E282D mutations	WAETGGMGLAVRQAPLI I PLKATSTPVS IKQYP MSREARLG I KPHI HRLLDQG I LVPCQSPWNTPL RPVKKPGTNDYRPVQDLREV NKRVEDI HPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPT LFDEALHRDLADFR I QHPDL I LLQYVDDL LLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT DARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLD TDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTN SRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLS I IHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
679	MMLV RTase with Q68R/Q79I/L99R/E282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS IKQYP MSREARLG I KPHI IRLLDQG I LVPCQSPWNTPL RPVKKPGTNDYRPVQDLREV NKRVEDI HPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPT LFDEALHRDLADFR I QHPDL I LLQYVDDL LLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT DARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT M GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLD TDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTN SRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLS I IHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
680	MMLV RTase with Q68R/Q79R/L99R/E282M mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI I PLKATSTPVS IKQYP MSREARLG I KPHI IRLLDQG I LVPCQSPWNTPL RPVKKPGTNDYRPVQDLREV NKRVEDI HPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPT LFDEALHRDLADFR I QHPDL I LLQYVDDL LLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT MARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK

		TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
681	MMLV RTase with Q68R/Q79R/L99R/E282W mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSIKQYP MSREARLGKPHIRRLDQGILVPCQSPWNTPL RPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFRIQHPDLILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGILLKEGQRWLTWARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
682	MMLV RTase with I61K/Q68R/Q79R/L99R/E2 82D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSKQYP MSREARLGKPHIRRLDQGILVPCQSPWNTPL RPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNST LFDEALHRDLADFRIQHPDLILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGILLKEGQRWLTWARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL

		PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
683	MMLV RTase with I61M/Q68R/Q79R/L99R/E 282D mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSMKQYP MSREARLG I KPHIRRLDQGILVPCQSPWNTPL RPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR IQHPDL ILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT DARETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLD TDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTN SRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
684	MMLV RTase with Q68I/Q79H/L99K/E282M mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSIKQYP MSIEARLG I KPHIRRLDQGILVPCQSPWNTPL KPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR IQHPDL ILLQYVDDLLLA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLG YLLKEGQRWLT MARKETVMGQPTPKT PRQLREFLG TAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLG PWRRPVAYL SKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLD TDRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGGS LLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTN SRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
685	MMLV RTase with I61M/Q68I/Q79H/L99K/E2 82M mutations	MTLNIEDEHRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLI IPLKATSTPVSMKQYP MSIEARLG I KPHIRRLDQGILVPCQSPWNTPL KPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPN PYNLLSGLPPSHQWYTVLDLKD AFFCLRLHPTS QPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPT LFDEALHRDLADFR IQHPDL ILLQYVDDLLLA

		TSELDCQQGTRALLQTLGNLGYRASAKKAQICQ KQVKYLGYYLLKEGQRWLTMARKETVMGQPTPKT PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK TGTLFNWGPDQQKAYQEIKQALLTAPALGLPDL TKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYL SKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLT GQPLVILAPHAVEALVKQPPDRWLSNARMTHYQ ALLLDTDTRVQFGPVVALNPATLLPLPEEGLQHN CLDILAEAHGTRPDLTDQPLPDADHTWYTGSS LLQEGQRKAGAAVTTETETEVIAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHI HGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKA AITETPDTSTLLIENSSPYTSEHF
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c. Expression and purification of MMLV RTase and mutant variants

A colony with the appropriate strain was used to inoculate TB media (200 mL) with kanamycin (0.05 mg/mL) and grown at 37°C until an OD of approximately 0.9 was achieved followed by cooling of the flask for 30 minutes at 4°C. Protein expression was induced by the addition of 1 M IPTG (100 µL), followed by growth at 18°C for 21 hours. Cells were harvested by spinning samples at 4,700 x g for 10 minutes.

Cell pellets were re-suspended in a lysis buffer (50 mM NaPO₄, pH 7.8, 5% glycerol, 300 mM NaCl, 10 mM imidazole, 5 mM DTT, 0.01% n-octyl-β-D-glucopyranoside, DNaseI, 10 mM CaCl₂, lysozyme (1 mg/mL), and protease inhibitor). The sample was lysed on an Avestin Emulsiflex C3 pre-chilled to 4°C at 15-20 kpsi with three passes. Cell debris was removed by centrifuging the lysate at 16,000 x g for 30 minutes at 4°C.

Cleared lysates were applied to a HisTrap HP column (Cytiva Life Sciences, Cat #17524701). The resin was equilibrated with MMLV His-Bind buffer (50 mM NaPO₄, pH 7.8, 5% glycerol, 0.3 M NaCl, 10 mM imidazole, 1 mM DTT and 0.01% IGEPAL-CA), followed by sample loading. The samples were washed with MMLV His-Bind buffer, followed by a 25% B wash (B = MMLV His Elution buffer = 50 mM NaPO₄, pH 7.8, 5% glycerol, 0.3 M NaCl, 250 mM imidazole, 1 mM DTT and 0.01% IGEPAL-CA). The sample was eluted with 100% B for 10 CVs in 45 mL fractions.

Purified proteins were applied to a HiTrap Heparin HP column (Cytiva Life Sciences, Cat #17040601). The resin was equilibrated with MMLV Heparin-Bind buffer (50 mM Tris HCl pH 8.5, 75 mM NaCl, 1 mM DTT, 5% glycerol and 0.01% IGEPAL-CA), followed by sample loading. The sample was washed with MLV Heparin Bind buffer, followed by a 25%

B wash (B = MLV Heparin Elution Buffer). The sample was eluted with 60% B for 10 CVs in 45 mL fractions.

Purified proteins were applied to a Bio-Scale™ Mini CHT™ Cartridge (Bio-Rad Laboratories, Cat #7324322). The resin was washed with 1 M NaOH, followed by equilibration with MMLV Heparin-Bind buffer and sample loading. The sample was washed with MLV Heparin Elution buffer, followed by MMLV Heparin Bind buffer. The sample was linearly eluted to 100% B2 (B2 = MMLV HA Elution Buffer = 250 mM KPO₄ pH 7.5, 1 mM DTT, 5% glycerol and 0.01% IGEPAL-CA) for 15 CVs in 5 mL fractions.

Fractions containing purified protein were pooled and dialyzed in MMLV Storage Buffer (50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1mM DTT, 50% (v/v) glycerol).

d. Evaluation of ability of purified MMLV RTase mutant variants to synthesize DNA by gene specific priming

MMLV RTase base construct and MMLV RTase mutant variants evaluated as described in Example 3. Temperatures were adjusted for both two-step and one-step reactions to 55 and 60°C, respectively. The two-step and one-step reactions for MMLV RTase base construct and MMLV RTase mutant variants were analyzed and reported by Ct output from the qPCR (*see* Tables 13 and 14).

Six of the seventeen MMLV RTase triple or more mutant variants were found to exhibit increased overall activity and thermostability as compared to the other MMLV RTase stacked mutant variants, and almost all of the MMLV RTase stacked mutant variants exhibited increased overall activity and thermostability as compared to the MMLV RTase base construct. The six MMLV RTase mutant variants that were found to exhibit the highest overall activity were Q68R/L99R, Q68R/Q79R/L99R, Q68R/Q79R/L99R/E282D, Q68R/Q79R/L99K/E282D, Q68R/Q79R/L99R/E282W, I61M/Q68R/Q79R/L99R/E282D and Q68I/Q79H/L99K/E282M.

Table 13. Two-Step cDNA synthesis by MMLV RT triple and more mutants. Data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV RT Variant	Concentration of RTase (nM)	Ct Mean	Ct Standard Deviation
MMLV-II	0.625	25.520	0.047
MMLV-II L99R/E282D	0.625	24.332	0.060
MMLV-II Q68R/L99R	0.625	22.207	0.097
MMLV-II Q79R/L99R	0.625	23.789	0.012
MMLV-II Q68R/Q79R	0.625	23.629	0.038

MMLV-II Q68R/L99R/E282D	0.625	22.855	0.079
MMLV-II Q79R/L99R/E282D	0.625	23.095	0.035
MMLV-II Q68R/Q79R/E282D	0.625	22.526	0.027
MMLV-II Q68R/Q79R/L99R	0.625	22.099	0.018
MMLV-II Q68R/Q79R/L99R/E282D	0.625	21.056	0.023
MMLV-II Q68R/Q79R/L99K/E282D	0.625	21.833	0.031
MMLV-II Q68R/Q79R/L99N/E282D	0.625	23.607	0.031
MMLV-II Q68I/Q79R/L99R/E282D	0.625	23.858	0.029
MMLV-II Q68K/Q79R/L99R/E282D	0.625	22.615	0.054
MMLV-II Q68R/Q79H/L99R/E282D	0.625	28.866	0.008
MMLV-II Q68R/Q79I/L99R/E282D	0.625	23.283	0.085
MMLV-II Q68R/Q79R/L99R/E282M	0.625	25.073	0.097
MMLV-II Q68R/Q79R/L99R/E282W	0.625	22.331	0.048
MMLV-II I61K/Q68R/Q79R/L99R/E282D	0.625	23.271	0.065
MMLV-II I61M/Q68R/Q79R/L99R/E282D	0.625	22.133	0.018
MMLV-II Q68I/Q79H/L99K/E282M	0.625	23.344	0.037
MMLV-II I61M/Q68I/Q79H/L99K/E282M	0.625	25.255	0.058
MMLV-II	2.5	22.154	0.052
MMLV-II L99R/E282D	2.5	21.501	0.054
MMLV-II Q68R/L99R	2.5	21.151	0.048
MMLV-II Q79R/L99R	2.5	21.229	0.163
MMLV-II Q68R/Q79R	2.5	21.228	0.054
MMLV-II Q68R/L99R/E282D	2.5	21.126	0.030
MMLV-II Q79R/L99R/E282D	2.5	21.418	0.033
MMLV-II Q68R/Q79R/E282D	2.5	21.011	0.052
MMLV-II Q68R/Q79R/L99R	2.5	20.953	0.041
MMLV-II Q68R/Q79R/L99R/E282D	2.5	21.113	0.108
MMLV-II Q68R/Q79R/L99K/E282D	2.5	20.906	0.081
MMLV-II Q68R/Q79R/L99N/E282D	2.5	21.196	0.029
MMLV-II Q68I/Q79R/L99R/E282D	2.5	21.369	0.009
MMLV-II Q68K/Q79R/L99R/E282D	2.5	20.960	0.030

MMLV-II Q68R/Q79H/L99R/E282D	2.5	26.167	0.038
MMLV-II Q68R/Q79I/L99R/E282D	2.5	21.012	0.056
MMLV-II Q68R/Q79R/L99R/E282M	2.5	21.277	0.036
MMLV-II Q68R/Q79R/L99R/E282W	2.5	20.944	0.020
MMLV-II I61K/Q68R/Q79R/L99R/E282D	2.5	21.320	0.009
MMLV-II I61M/Q68R/Q79R/L99R/E282D	2.5	21.095	0.013
MMLV-II Q68I/Q79H/L99K/E282M	2.5	21.329	0.047
MMLV-II I61M/Q68I/Q79H/L99K/E282M	2.5	22.159	0.031
MMLV-II	10	21.575	0.101
MMLV-II L99R/E282D	10	21.546	0.041
MMLV-II Q68R/L99R	10	21.343	0.021
MMLV-II Q79R/L99R	10	21.387	0.016
MMLV-II Q68R/Q79R	10	21.147	0.032
MMLV-II Q68R/L99R/E282D	10	21.265	0.076
MMLV-II Q79R/L99R/E282D	10	21.250	0.036
MMLV-II Q68R/Q79R/E282D	10	21.135	0.015
MMLV-II Q68R/Q79R/L99R	10	21.051	0.036
MMLV-II Q68R/Q79R/L99R/E282D	10	21.159	0.065
MMLV-II Q68R/Q79R/L99K/E282D	10	21.056	0.032
MMLV-II Q68R/Q79R/L99N/E282D	10	21.180	0.052
MMLV-II Q68I/Q79R/L99R/E282D	10	21.068	0.069
MMLV-II Q68K/Q79R/L99R/E282D	10	21.065	0.053
MMLV-II Q68R/Q79H/L99R/E282D	10	21.683	0.075
MMLV-II Q68R/Q79I/L99R/E282D	10	21.152	0.064
MMLV-II Q68R/Q79R/L99R/E282M	10	21.029	0.055
MMLV-II Q68R/Q79R/L99R/E282W	10	21.214	0.052
MMLV-II I61K/Q68R/Q79R/L99R/E282D	10	21.391	0.051
MMLV-II I61M/Q68R/Q79R/L99R/E282D	10	21.307	0.038
MMLV-II Q68I/Q79H/L99K/E282M	10	21.583	0.019

MMLV-II I61M/Q68I/Q79H/L99K/E282M	10	21.759	0.029
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Table 14. One-Step cDNA synthesis by MMLV RT triple and more mutants. Data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV RT Variant	Concentration of RTase (nM)	Ct Mean	Ct Standard Deviation
MMLV-II	0.625	22.153	0.122
MMLV-II L99R/E282D	0.625	21.713	0.111
MMLV-II Q68R/L99R	0.625	21.334	0.167
MMLV-II Q79R/L99R	0.625	21.398	0.069
MMLV-II Q68R/Q79R	0.625	21.546	0.096
MMLV-II Q68R/L99R/E282D	0.625	21.112	0.149
MMLV-II Q79R/L99R/E282D	0.625	21.260	0.104
MMLV-II Q68R/Q79R/E282D	0.625	21.014	0.102
MMLV-II Q68R/Q79R/L99R	0.625	20.338	0.042
MMLV-II Q68R/Q79R/L99R/E282D	0.625	19.537	0.120
MMLV-II Q68R/Q79R/L99K/E282D	0.625	20.516	0.131
MMLV-II Q68R/Q79R/L99N/E282D	0.625	20.960	0.023
MMLV-II Q68I/Q79R/L99R/E282D	0.625	21.325	0.088
MMLV-II Q68K/Q79R/L99R/E282D	0.625	20.602	0.038
MMLV-II Q68R/Q79H/L99R/E282D	0.625	23.889	0.042
MMLV-II Q68R/Q79I/L99R/E282D	0.625	21.375	0.035
MMLV-II Q68R/Q79R/L99R/E282M	0.625	21.805	0.054
MMLV-II Q68R/Q79R/L99R/E282W	0.625	20.229	0.085
MMLV-II I61K/Q68R/Q79R/L99R/E282D	0.625	20.972	0.037
MMLV-II I61M/Q68R/Q79R/L99R/E282D	0.625	20.225	0.042
MMLV-II Q68I/Q79H/L99K/E282M	0.625	20.578	0.061
MMLV-II I61M/Q68I/Q79H/L99K/E282M	0.625	21.107	0.101
MMLV-II	2.5	20.874	0.042
MMLV-II L99R/E282D	2.5	19.679	0.047
MMLV-II Q68R/L99R	2.5	19.152	0.024
MMLV-II Q79R/L99R	2.5	19.202	0.091
MMLV-II Q68R/Q79R	2.5	19.506	0.010
MMLV-II Q68R/L99R/E282D	2.5	19.142	0.060

MMLV-II Q79R/L99R/E282D	2.5	19.301	0.004
MMLV-II Q68R/Q79R/E282D	2.5	19.023	0.041
MMLV-II Q68R/Q79R/L99R	2.5	18.312	0.041
MMLV-II Q68R/Q79R/L99R/E282D	2.5	17.867	0.099
MMLV-II Q68R/Q79R/L99K/E282D	2.5	18.591	0.036
MMLV-II Q68R/Q79R/L99N/E282D	2.5	19.123	0.097
MMLV-II Q68I/Q79R/L99R/E282D	2.5	19.553	0.076
MMLV-II Q68K/Q79R/L99R/E282D	2.5	18.771	0.113
MMLV-II Q68R/Q79H/L99R/E282D	2.5	21.911	0.048
MMLV-II Q68R/Q79I/L99R/E282D	2.5	19.298	0.146
MMLV-II Q68R/Q79R/L99R/E282M	2.5	19.621	0.027
MMLV-II Q68R/Q79R/L99R/E282W	2.5	18.219	0.103
MMLV-II I61K/Q68R/Q79R/L99R/E282D	2.5	18.846	0.056
MMLV-II I61M/Q68R/Q79R/L99R/E282D	2.5	18.500	0.042
MMLV-II Q68I/Q79H/L99K/E282M	2.5	18.752	0.148
MMLV-II I61M/Q68I/Q79H/L99K/E282M	2.5	19.445	0.098
MMLV-II	10	18.239	0.025
MMLV-II L99R/E282D	10	17.293	0.021
MMLV-II Q68R/L99R	10	17.144	0.032
MMLV-II Q79R/L99R	10	17.324	0.016
MMLV-II Q68R/Q79R	10	17.123	0.072
MMLV-II Q68R/L99R/E282D	10	17.082	0.088
MMLV-II Q79R/L99R/E282D	10	17.353	0.068
MMLV-II Q68R/Q79R/E282D	10	17.111	0.036
MMLV-II Q68R/Q79R/L99R	10	16.562	0.101
MMLV-II Q68R/Q79R/L99R/E282D	10	16.492	0.066
MMLV-II Q68R/Q79R/L99K/E282D	10	17.027	0.054
MMLV-II Q68R/Q79R/L99N/E282D	10	17.335	0.080
MMLV-II Q68I/Q79R/L99R/E282D	10	17.726	0.055
MMLV-II Q68K/Q79R/L99R/E282D	10	17.144	0.140
MMLV-II	10	19.772	0.064

Q68R/Q79H/L99R/E282D			
MMLV-II	10		
Q68R/Q79I/L99R/E282D		17.424	0.020
MMLV-II	10		
Q68R/Q79R/L99R/E282M		17.624	0.014
MMLV-II	10		
Q68R/Q79R/L99R/E282W		16.629	0.080
MMLV-II	10		
I61K/Q68R/Q79R/L99R/E282D		16.903	0.022
MMLV-II	10		
I61M/Q68R/Q79R/L99R/E282D		16.803	0.028
MMLV-II	10		
Q68I/Q79H/L99K/E282M		16.894	0.056
MMLV-II	10		
I61M/Q68I/Q79H/L99K/E282M		17.509	0.058

e. Evaluation of ability of purified MMLV RTase mutant variants to synthesize DNA by oligo-dT or random priming

MMLV RTase base construct and MMLV RTase mutant variants evaluated as described in Example 3. Oligo-dT or random hexamer priming conditions were adjusted for the two-step reactions and RTase concentration was normalized to 31 nM. The two-step reactions for MMLV RTase base construct and MMLV RTase mutant variants were analyzed and reported by Ct output from the qPCR (*see* Tables 15 and 16).

Nine of the seventeen MMLV RTase triple or more mutant variants were found to exhibit increased overall activity and thermostability as compared to the other MMLV RTase stacked mutant variants, and almost all of the MMLV RTase stacked mutant variants exhibited increased overall activity and thermostability as compared to the MMLV RTase base construct. The nine MMLV RTase mutant variants that were found to exhibit the highest overall activity were Q79R/L99R/E282D, Q68R/Q79R/L99R, Q68R/Q79R/L99R/E282D, Q68R/Q79R/L99K/E282D, Q68R/Q79R/L99N/E282D, Q68K/Q79R/L99R/E282D, Q68R/Q79R/L99R/E282M, I61K/Q68R/Q79R/L99R/E282D and I61M/Q68R/Q79R/L99R/E282D.

Table 15. Two-Step cDNA synthesis by MMLV RT triple and more mutants by Oligo-dT priming. Data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV RT Variant	Temperature of Reaction (°C)	Ct Mean	Ct Standard Deviation
MMLV-II	42	25.165	0.057
MMLV-II L99R/E282D	42	25.287	0.062

MMLV-II Q68R/L99R	42	25.026	0.035
MMLV-II Q79R/L99R	42	24.932	0.032
MMLV-II Q68R/Q79R	42	25.002	0.076
MMLV-II Q68R/L99R/E282D	42	24.964	0.068
MMLV-II Q79R/L99R/E282D	42	24.822	0.106
MMLV-II Q68R/Q79R/E282D	42	24.905	0.134
MMLV-II Q68R/Q79R/L99R	42	24.673	0.131
MMLV-II Q68R/Q79R/L99R/E282D	42	24.523	0.111
MMLV-II Q68R/Q79R/L99K/E282D	42	24.677	0.076
MMLV-II Q68R/Q79R/L99N/E282D	42	24.635	0.087
MMLV-II Q68I/Q79R/L99R/E282D	42	25.010	0.074
MMLV-II Q68K/Q79R/L99R/E282D	42	24.676	0.066
MMLV-II Q68R/Q79H/L99R/E282D	42	28.929	0.021
MMLV-II Q68R/Q79I/L99R/E282D	42	24.932	0.039
MMLV-II Q68R/Q79R/L99R/E282M	42	24.900	0.113
MMLV-II Q68R/Q79R/L99R/E282W	42	24.967	0.091
MMLV-II I61K/Q68R/Q79R/L99R/E282D	42	24.597	0.076
MMLV-II I61M/Q68R/Q79R/L99R/E282D	42	24.833	0.007
MMLV-II Q68I/Q79H/L99K/E282M	42	25.440	0.048
MMLV-II I61M/Q68I/Q79H/L99K/E282M	42	25.679	0.050
MMLV-II	55	34.223	0.406
MMLV-II L99R/E282D	55	34.732	3.729
MMLV-II Q68R/L99R	55	31.509	0.169
MMLV-II Q79R/L99R	55	31.831	0.019
MMLV-II Q68R/Q79R	55	32.633	1.094
MMLV-II Q68R/L99R/E282D	55	32.089	0.075
MMLV-II Q79R/L99R/E282D	55	32.134	0.081
MMLV-II Q68R/Q79R/E282D	55	34.639	3.791
MMLV-II Q68R/Q79R/L99R	55	29.559	0.029
MMLV-II Q68R/Q79R/L99R/E282D	55	28.013	0.136
MMLV-II Q68R/Q79R/L99K/E282D	55	29.712	0.090
MMLV-II Q68R/Q79R/L99N/E282D	55	30.442	0.224
MMLV-II	55	32.857	0.378

Q68I/Q79R/L99R/E282D			
MMLV-II Q68K/Q79R/L99R/E282D	55	31.186	0.630
MMLV-II Q68R/Q79H/L99R/E282D	55	37.338	1.882
MMLV-II Q68R/Q79I/L99R/E282D	55	31.830	0.120
MMLV-II Q68R/Q79R/L99R/E282M	55	31.682	0.181
MMLV-II Q68R/Q79R/L99R/E282W	55	32.256	0.228
MMLV-II I61K/Q68R/Q79R/L99R/E282D	55	30.362	0.129
MMLV-II I61M/Q68R/Q79R/L99R/E282D	55	31.473	0.070
MMLV-II Q68I/Q79H/L99K/E282M	55	32.892	0.286
MMLV-II I61M/Q68I/Q79H/L99K/E282M	55	33.872	0.131

Table 16. Two-Step cDNA synthesis by MMLV RT triple and more mutants by random hexamer priming. Data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV RT Variant	Temperature of Reaction (°C)	Ct Mean	Ct Standard Deviation
MMLV-II	42	24.675	0.054
MMLV-II L99R/E282D	42	24.864	0.043
MMLV-II Q68R/L99R	42	24.577	0.066
MMLV-II Q79R/L99R	42	24.630	0.103
MMLV-II Q68R/Q79R	42	24.496	0.050
MMLV-II Q68R/L99R/E282D	42	24.549	0.059
MMLV-II Q79R/L99R/E282D	42	24.625	0.013
MMLV-II Q68R/Q79R/E282D	42	24.623	0.083
MMLV-II Q68R/Q79R/L99R	42	24.494	0.070
MMLV-II Q68R/Q79R/L99R/E282D	42	24.422	0.035
MMLV-II Q68R/Q79R/L99K/E282D	42	24.517	0.066
MMLV-II Q68R/Q79R/L99N/E282D	42	24.324	0.059
MMLV-II Q68I/Q79R/L99R/E282D	42	24.488	0.070
MMLV-II Q68K/Q79R/L99R/E282D	42	24.501	0.041
MMLV-II Q68R/Q79H/L99R/E282D	42	26.574	0.029
MMLV-II Q68R/Q79I/L99R/E282D	42	24.496	0.055

MMLV-II Q68R/Q79R/L99R/E282M	42	24.382	0.043
MMLV-II Q68R/Q79R/L99R/E282W	42	24.617	0.109
MMLV-II I61K/Q68R/Q79R/L99R/E282D	42	24.391	0.045
MMLV-II I61M/Q68R/Q79R/L99R/E282D	42	24.426	0.028
MMLV-II Q68I/Q79H/L99K/E282M	42	24.660	0.027
MMLV-II I61M/Q68I/Q79H/L99K/E282M	42	24.949	0.052
MMLV-II	55	32.082	0.095
MMLV-II L99R/E282D	55	31.612	0.190
MMLV-II Q68R/L99R	55	30.349	0.041
MMLV-II Q79R/L99R	55	30.494	0.094
MMLV-II Q68R/Q79R	55	29.735	0.153
MMLV-II Q68R/L99R/E282D	55	30.724	0.045
MMLV-II Q79R/L99R/E282D	55	30.774	0.152
MMLV-II Q68R/Q79R/E282D	55	30.232	0.079
MMLV-II Q68R/Q79R/L99R	55	28.270	0.340
MMLV-II Q68R/Q79R/L99R/E282D	55	26.673	0.143
MMLV-II Q68R/Q79R/L99K/E282D	55	28.258	0.018
MMLV-II Q68R/Q79R/L99N/E282D	55	28.973	0.116
MMLV-II Q68I/Q79R/L99R/E282D	55	31.617	0.071
MMLV-II Q68K/Q79R/L99R/E282D	55	28.994	0.110
MMLV-II Q68R/Q79H/L99R/E282D	55	35.664	0.695
MMLV-II Q68R/Q79I/L99R/E282D	55	30.265	0.116
MMLV-II Q68R/Q79R/L99R/E282M	55	29.765	0.059
MMLV-II Q68R/Q79R/L99R/E282W	55	30.535	0.424
MMLV-II I61K/Q68R/Q79R/L99R/E282D	55	28.878	0.038
MMLV-II I61M/Q68R/Q79R/L99R/E282D	55	29.778	0.081
MMLV-II Q68I/Q79H/L99K/E282M	55	31.836	0.222
MMLV-II I61M/Q68I/Q79H/L99K/E282M	55	31.984	0.223

f. Evaluation of ability of purified MMLV RTase mutant variants to synthesize DNA over a wide range of temperatures

MMLV RTase base construct and MMLV RTase mutant variants evaluated as described in Example 3. Oligo-dT or random hexamer priming conditions and reaction temperatures were adjusted for the two-step reactions and RTase concentration was normalized to 31 nM. The two-step reactions for MMLV RTase base construct and MMLV RTase mutant variants were analyzed and reported by Ct output from the qPCR (*see* Tables 17 and 18).

Six of the nine MMLV RTase triple or more mutant variants were found to exhibit high overall activity as compared to the other MMLV RTase stacked mutant variants over a wide range of temperatures, spanning from 37.0 to 65 °C, regardless of which priming method used. All of the MMLV RTase stacked mutant variants exhibited increased overall activity and thermostability as compared to the MMLV RTase base construct. The six MMLV RTase mutant variants that were found to exhibit the highest overall activity at a wide range of temperatures were Q68R/Q79R/L99R, Q68R/Q79R/L99R/E282D, Q68R/Q79R/L99K/E282D, Q68R/Q79R/L99N/E282D, I61K/Q68R/Q79R/L99R/E282D and I61M/Q68R/Q79R/L99R/E282D.

Table 17. Two-Step cDNA synthesis by MMLV RT triple and more mutants by Oligo-dT priming. Data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV RT Variant	Temperature of Reaction (°C)	Ct Mean	Ct Standard Deviation
MMLV-II	37.0	26.593	0.020
MMLV-II Q79R/L99R/E282D	37.0	25.713	0.024
MMLV-II Q68R/Q79R/L99R	37.0	25.164	0.059
MMLV-II Q68R/Q79R/L99R/E282D	37.0	25.163	0.035
MMLV-II Q68R/Q79R/L99K/E282D	37.0	25.135	0.078
MMLV-II Q68R/Q79R/L99N/E282D	37.0	25.693	0.048
MMLV-II Q68K/Q79R/L99R/E282D	37.0	25.491	0.062
MMLV-II Q68R/Q79R/L99R/E282M	37.0	25.450	0.083
MMLV-II I61K/Q68R/Q79R/L99R/E282D	37.0	25.094	0.071
MMLV-II I61M/Q68R/Q79R/L99R/E282D	37.0	25.356	0.034

MMLV-II	37.8	26.623	0.062
MMLV-II Q79R/L99R/E282D	37.8	25.516	0.078
MMLV-II Q68R/Q79R/L99R	37.8	25.251	0.094
MMLV-II Q68R/Q79R/L99R/E282D	37.8	24.987	0.050
MMLV-II Q68R/Q79R/L99K/E282D	37.8	25.093	0.084
MMLV-II Q68R/Q79R/L99N/E282D	37.8	25.273	0.095
MMLV-II Q68K/Q79R/L99R/E282D	37.8	25.310	0.079
MMLV-II Q68R/Q79R/L99R/E282M	37.8	25.545	0.044
MMLV-II I61K/Q68R/Q79R/L99R/E282D	37.8	25.144	0.196
MMLV-II I61M/Q68R/Q79R/L99R/E282D	37.8	25.302	0.035
MMLV-II	39.5	26.430	0.074
MMLV-II Q79R/L99R/E282D	39.5	25.067	0.026
MMLV-II Q68R/Q79R/L99R	39.5	25.138	0.050
MMLV-II Q68R/Q79R/L99R/E282D	39.5	24.788	0.022
MMLV-II Q68R/Q79R/L99K/E282D	39.5	24.842	0.071
MMLV-II Q68R/Q79R/L99N/E282D	39.5	24.892	0.042
MMLV-II Q68K/Q79R/L99R/E282D	39.5	25.047	0.038
MMLV-II Q68R/Q79R/L99R/E282M	39.5	25.249	0.081
MMLV-II I61K/Q68R/Q79R/L99R/E282D	39.5	24.845	0.130
MMLV-II I61M/Q68R/Q79R/L99R/E282D	39.5	25.130	0.072
MMLV-II	42.0	25.485	0.052
MMLV-II Q79R/L99R/E282D	42.0	24.941	0.024
MMLV-II Q68R/Q79R/L99R	42.0	24.848	0.101
MMLV-II Q68R/Q79R/L99R/E282D	42.0	24.802	0.009
MMLV-II Q68R/Q79R/L99K/E282D	42.0	24.805	0.008
MMLV-II Q68R/Q79R/L99N/E282D	42.0	24.744	0.076
MMLV-II Q68K/Q79R/L99R/E282D	42.0	24.893	0.073
MMLV-II Q68R/Q79R/L99R/E282M	42.0	24.968	0.031
MMLV-II I61K/Q68R/Q79R/L99R/E282D	42.0	24.933	0.088

MMLV-II I61M/Q68R/Q79R/L99R/E282D	42.0	24.821	0.045
MMLV-II	45.2	25.776	0.028
MMLV-II Q79R/L99R/E282D	45.2	24.902	0.034
MMLV-II Q68R/Q79R/L99R	45.2	24.792	0.055
MMLV-II Q68R/Q79R/L99R/E282D	45.2	24.705	0.092
MMLV-II Q68R/Q79R/L99K/E282D	45.2	24.791	0.009
MMLV-II Q68R/Q79R/L99N/E282D	45.2	24.890	0.071
MMLV-II Q68K/Q79R/L99R/E282D	45.2	25.420	0.101
MMLV-II Q68R/Q79R/L99R/E282M	45.2	25.196	0.086
MMLV-II I61K/Q68R/Q79R/L99R/E282D	45.2	24.823	0.079
MMLV-II I61M/Q68R/Q79R/L99R/E282D	45.2	24.720	0.006
MMLV-II	47.8	27.932	0.049
MMLV-II Q79R/L99R/E282D	47.8	24.858	0.063
MMLV-II Q68R/Q79R/L99R	47.8	24.685	0.095
MMLV-II Q68R/Q79R/L99R/E282D	47.8	24.689	0.067
MMLV-II Q68R/Q79R/L99K/E282D	47.8	24.620	0.072
MMLV-II Q68R/Q79R/L99N/E282D	47.8	24.780	0.039
MMLV-II Q68K/Q79R/L99R/E282D	47.8	24.855	0.018
MMLV-II Q68R/Q79R/L99R/E282M	47.8	24.961	0.040
MMLV-II I61K/Q68R/Q79R/L99R/E282D	47.8	24.681	0.076
MMLV-II I61M/Q68R/Q79R/L99R/E282D	47.8	24.759	0.055
MMLV-II	49.2	30.393	0.118
MMLV-II Q79R/L99R/E282D	49.2	24.974	0.090
MMLV-II Q68R/Q79R/L99R	49.2	24.794	0.056
MMLV-II Q68R/Q79R/L99R/E282D	49.2	24.720	0.100
MMLV-II Q68R/Q79R/L99K/E282D	49.2	25.007	0.096
MMLV-II Q68R/Q79R/L99N/E282D	49.2	25.304	0.147
MMLV-II Q68K/Q79R/L99R/E282D	49.2	25.273	0.066
MMLV-II Q68R/Q79R/L99R/E282M	49.2	25.560	0.019

MMLV-II I61K/Q68R/Q79R/L99R/E282D	49.2	24.719	0.177
MMLV-II I61M/Q68R/Q79R/L99R/E282D	49.2	25.123	0.034
MMLV-II	50.0	30.870	0.210
MMLV-II Q79R/L99R/E282D	50.0	26.677	0.090
MMLV-II Q68R/Q79R/L99R	50.0	25.381	0.049
MMLV-II Q68R/Q79R/L99R/E282D	50.0	24.820	0.064
MMLV-II Q68R/Q79R/L99K/E282D	50.0	25.348	0.098
MMLV-II Q68R/Q79R/L99N/E282D	50.0	25.287	0.064
MMLV-II Q68K/Q79R/L99R/E282D	50.0	25.208	0.085
MMLV-II Q68R/Q79R/L99R/E282M	50.0	25.790	0.051
MMLV-II I61K/Q68R/Q79R/L99R/E282D	50.0	24.840	0.071
MMLV-II I61M/Q68R/Q79R/L99R/E282D	50.0	25.317	0.042
MMLV-II	51.0	27.914	0.002
MMLV-II Q79R/L99R/E282D	51.0	25.561	0.069
MMLV-II Q68R/Q79R/L99R	51.0	25.225	0.069
MMLV-II Q68R/Q79R/L99R/E282D	51.0	24.726	0.034
MMLV-II Q68R/Q79R/L99K/E282D	51.0	25.324	0.071
MMLV-II Q68R/Q79R/L99N/E282D	51.0	25.157	0.062
MMLV-II Q68K/Q79R/L99R/E282D	51.0	25.275	0.039
MMLV-II Q68R/Q79R/L99R/E282M	51.0	25.938	0.095
MMLV-II I61K/Q68R/Q79R/L99R/E282D	51.0	25.821	0.072
MMLV-II I61M/Q68R/Q79R/L99R/E282D	51.0	25.053	0.044
MMLV-II	51.9	28.602	0.059
MMLV-II Q79R/L99R/E282D	51.9	25.975	0.024
MMLV-II Q68R/Q79R/L99R	51.9	25.256	0.075
MMLV-II Q68R/Q79R/L99R/E282D	51.9	24.903	0.050
MMLV-II Q68R/Q79R/L99K/E282D	51.9	25.163	0.169
MMLV-II Q68R/Q79R/L99N/E282D	51.9	25.272	0.011
MMLV-II Q68K/Q79R/L99R/E282D	51.9	25.491	0.075

MMLV-II Q68R/Q79R/L99R/E282M	51.9	25.878	0.038
MMLV-II I61K/Q68R/Q79R/L99R/E282D	51.9	26.071	0.044
MMLV-II I61M/Q68R/Q79R/L99R/E282D	51.9	25.419	0.067
MMLV-II	53.8	26.412	0.082
MMLV-II Q79R/L99R/E282D	53.8	25.558	0.063
MMLV-II Q68R/Q79R/L99R	53.8	24.969	0.065
MMLV-II Q68R/Q79R/L99R/E282D	53.8	25.356	0.063
MMLV-II Q68R/Q79R/L99K/E282D	53.8	25.460	0.056
MMLV-II Q68R/Q79R/L99N/E282D	53.8	25.769	0.118
MMLV-II Q68K/Q79R/L99R/E282D	53.8	26.251	0.103
MMLV-II Q68R/Q79R/L99R/E282M	53.8	26.310	0.174
MMLV-II I61K/Q68R/Q79R/L99R/E282D	53.8	25.701	0.106
MMLV-II I61M/Q68R/Q79R/L99R/E282D	53.8	26.412	0.082
MMLV-II	56.5	29.343	0.085
MMLV-II Q79R/L99R/E282D	56.5	26.885	0.083
MMLV-II Q68R/Q79R/L99R	56.5	25.736	0.015
MMLV-II Q68R/Q79R/L99R/E282D	56.5	25.223	0.016
MMLV-II Q68R/Q79R/L99K/E282D	56.5	25.900	0.039
MMLV-II Q68R/Q79R/L99N/E282D	56.5	25.930	0.031
MMLV-II Q68K/Q79R/L99R/E282D	56.5	25.869	0.204
MMLV-II Q68R/Q79R/L99R/E282M	56.5	26.622	0.067
MMLV-II I61K/Q68R/Q79R/L99R/E282D	56.5	25.817	0.089
MMLV-II I61M/Q68R/Q79R/L99R/E282D	56.5	26.290	0.009
MMLV-II	59.9	29.693	0.047
MMLV-II Q79R/L99R/E282D	59.9	27.820	0.014
MMLV-II Q68R/Q79R/L99R	59.9	26.069	0.057
MMLV-II Q68R/Q79R/L99R/E282D	59.9	25.374	0.061
MMLV-II Q68R/Q79R/L99K/E282D	59.9	26.066	0.053
MMLV-II Q68R/Q79R/L99N/E282D	59.9	25.873	0.018

MMLV-II Q68K/Q79R/L99R/E282D	59.9	26.278	0.073
MMLV-II Q68R/Q79R/L99R/E282M	59.9	27.068	0.075
MMLV-II I61K/Q68R/Q79R/L99R/E282D	59.9	26.863	0.025
MMLV-II I61M/Q68R/Q79R/L99R/E282D	59.9	26.176	0.072
MMLV-II	62.6	29.731	0.092
MMLV-II Q79R/L99R/E282D	62.6	27.161	0.035
MMLV-II Q68R/Q79R/L99R	62.6	25.929	0.026
MMLV-II Q68R/Q79R/L99R/E282D	62.6	25.303	0.074
MMLV-II Q68R/Q79R/L99K/E282D	62.6	25.907	0.003
MMLV-II Q68R/Q79R/L99N/E282D	62.6	26.145	0.053
MMLV-II Q68K/Q79R/L99R/E282D	62.6	26.181	0.056
MMLV-II Q68R/Q79R/L99R/E282M	62.6	27.134	0.015
MMLV-II I61K/Q68R/Q79R/L99R/E282D	62.6	26.025	0.178
MMLV-II I61M/Q68R/Q79R/L99R/E282D	62.6	26.304	0.041
MMLV-II	64.2	26.809	0.080
MMLV-II Q79R/L99R/E282D	64.2	27.325	0.038
MMLV-II Q68R/Q79R/L99R	64.2	26.131	0.018
MMLV-II Q68R/Q79R/L99R/E282D	64.2	25.542	0.135
MMLV-II Q68R/Q79R/L99K/E282D	64.2	26.408	0.093
MMLV-II Q68R/Q79R/L99N/E282D	64.2	26.734	0.040
MMLV-II Q68K/Q79R/L99R/E282D	64.2	30.589	0.128
MMLV-II Q68R/Q79R/L99R/E282M	64.2	26.262	0.090
MMLV-II I61K/Q68R/Q79R/L99R/E282D	64.2	27.594	0.118
MMLV-II I61M/Q68R/Q79R/L99R/E282D	64.2	27.062	0.051
MMLV-II	65.0	30.277	0.050
MMLV-II Q79R/L99R/E282D	65.0	27.119	0.065
MMLV-II Q68R/Q79R/L99R	65.0	26.078	0.025
MMLV-II Q68R/Q79R/L99R/E282D	65.0	25.583	0.068
MMLV-II Q68R/Q79R/L99K/E282D	65.0	25.906	0.080

MMLV-II Q68R/Q79R/L99N/E282D	65.0	26.943	0.058
MMLV-II Q68K/Q79R/L99R/E282D	65.0	26.413	0.067
MMLV-II Q68R/Q79R/L99R/E282M	65.0	28.233	0.075
MMLV-II I61K/Q68R/Q79R/L99R/E282D	65.0	25.778	0.129
MMLV-II I61M/Q68R/Q79R/L99R/E282D	65.0	27.345	0.015

Table 18. Two-Step cDNA synthesis by MMLV RT triple and more mutants by random hexamer priming. Data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV RT Variant	Temperature of Reaction (°C)	Ct Mean	Ct Standard Deviation
MMLV-II	37.0	25.827	0.120
MMLV-II Q79R/L99R/E282D	37.0	25.616	0.094
MMLV-II Q68R/Q79R/L99R	37.0	24.747	0.041
MMLV-II Q68R/Q79R/L99R/E282D	37.0	24.595	0.034
MMLV-II Q68R/Q79R/L99K/E282D	37.0	24.917	0.078
MMLV-II Q68R/Q79R/L99N/E282D	37.0	24.817	0.024
MMLV-II Q68K/Q79R/L99R/E282D	37.0	24.757	0.032
MMLV-II Q68R/Q79R/L99R/E282M	37.0	24.754	0.062
MMLV-II I61K/Q68R/Q79R/L99R/E282D	37.0	24.883	0.106
MMLV-II I61M/Q68R/Q79R/L99R/E282D	37.0	24.776	0.028
MMLV-II	37.8	25.609	0.038
MMLV-II Q79R/L99R/E282D	37.8	25.300	0.061
MMLV-II Q68R/Q79R/L99R	37.8	24.822	0.037
MMLV-II Q68R/Q79R/L99R/E282D	37.8	24.690	0.044
MMLV-II Q68R/Q79R/L99K/E282D	37.8	24.884	0.033
MMLV-II Q68R/Q79R/L99N/E282D	37.8	24.665	0.022
MMLV-II Q68K/Q79R/L99R/E282D	37.8	24.846	0.021
MMLV-II Q68R/Q79R/L99R/E282M	37.8	24.882	0.043
MMLV-II I61K/Q68R/Q79R/L99R/E282D	37.8	24.846	0.059

MMLV-II I61M/Q68R/Q79R/L99R/E282D	37.8	24.723	0.023
MMLV-II	39.5	25.455	0.020
MMLV-II Q79R/L99R/E282D	39.5	24.790	0.109
MMLV-II Q68R/Q79R/L99R	39.5	24.712	0.050
MMLV-II Q68R/Q79R/L99R/E282D	39.5	24.543	0.005
MMLV-II Q68R/Q79R/L99K/E282D	39.5	24.714	0.035
MMLV-II Q68R/Q79R/L99N/E282D	39.5	24.520	0.084
MMLV-II Q68K/Q79R/L99R/E282D	39.5	24.752	0.047
MMLV-II Q68R/Q79R/L99R/E282M	39.5	24.850	0.054
MMLV-II I61K/Q68R/Q79R/L99R/E282D	39.5	24.698	0.059
MMLV-II I61M/Q68R/Q79R/L99R/E282D	39.5	24.682	0.024
MMLV-II	42.0	25.136	0.034
MMLV-II Q79R/L99R/E282D	42.0	24.760	0.052
MMLV-II Q68R/Q79R/L99R	42.0	24.637	0.037
MMLV-II Q68R/Q79R/L99R/E282D	42.0	24.449	0.008
MMLV-II Q68R/Q79R/L99K/E282D	42.0	24.650	0.068
MMLV-II Q68R/Q79R/L99N/E282D	42.0	24.477	0.055
MMLV-II Q68K/Q79R/L99R/E282D	42.0	24.624	0.029
MMLV-II Q68R/Q79R/L99R/E282M	42.0	24.627	0.044
MMLV-II I61K/Q68R/Q79R/L99R/E282D	42.0	24.718	0.083
MMLV-II I61M/Q68R/Q79R/L99R/E282D	42.0	24.532	0.021
MMLV-II	45.2	25.079	0.017
MMLV-II Q79R/L99R/E282D	45.2	24.624	0.026
MMLV-II Q68R/Q79R/L99R	45.2	24.525	0.021
MMLV-II Q68R/Q79R/L99R/E282D	45.2	24.430	0.014
MMLV-II Q68R/Q79R/L99K/E282D	45.2	24.525	0.037
MMLV-II Q68R/Q79R/L99N/E282D	45.2	34.853	0.705
MMLV-II Q68K/Q79R/L99R/E282D	45.2	24.653	0.055
MMLV-II Q68R/Q79R/L99R/E282M	45.2	24.552	0.060

MMLV-II I61K/Q68R/Q79R/L99R/E282D	45.2	24.595	0.027
MMLV-II I61M/Q68R/Q79R/L99R/E282D	45.2	24.493	0.016
MMLV-II	47.8	25.346	0.007
MMLV-II Q79R/L99R/E282D	47.8	24.521	0.097
MMLV-II Q68R/Q79R/L99R	47.8	24.605	0.018
MMLV-II Q68R/Q79R/L99R/E282D	47.8	24.333	0.107
MMLV-II Q68R/Q79R/L99K/E282D	47.8	24.516	0.043
MMLV-II Q68R/Q79R/L99N/E282D	47.8	24.527	0.026
MMLV-II Q68K/Q79R/L99R/E282D	47.8	24.539	0.064
MMLV-II Q68R/Q79R/L99R/E282M	47.8	24.631	0.019
MMLV-II I61K/Q68R/Q79R/L99R/E282D	47.8	24.227	0.260
MMLV-II I61M/Q68R/Q79R/L99R/E282D	47.8	24.441	0.030
MMLV-II	49.2	25.791	0.064
MMLV-II Q79R/L99R/E282D	49.2	24.700	0.033
MMLV-II Q68R/Q79R/L99R	49.2	24.658	0.008
MMLV-II Q68R/Q79R/L99R/E282D	49.2	24.471	0.069
MMLV-II Q68R/Q79R/L99K/E282D	49.2	24.590	0.024
MMLV-II Q68R/Q79R/L99N/E282D	49.2	24.482	0.099
MMLV-II Q68K/Q79R/L99R/E282D	49.2	24.549	0.028
MMLV-II Q68R/Q79R/L99R/E282M	49.2	24.753	0.030
MMLV-II I61K/Q68R/Q79R/L99R/E282D	49.2	24.499	0.157
MMLV-II I61M/Q68R/Q79R/L99R/E282D	49.2	24.559	0.033
MMLV-II	50.0	26.267	0.025
MMLV-II Q79R/L99R/E282D	50.0	24.729	0.047
MMLV-II Q68R/Q79R/L99R	50.0	24.462	0.040
MMLV-II Q68R/Q79R/L99R/E282D	50.0	24.412	0.035
MMLV-II Q68R/Q79R/L99K/E282D	50.0	24.438	0.090
MMLV-II Q68R/Q79R/L99N/E282D	50.0	24.509	0.050
MMLV-II Q68K/Q79R/L99R/E282D	50.0	24.405	0.059

MMLV-II Q68R/Q79R/L99R/E282M	50.0	24.547	0.041
MMLV-II I61K/Q68R/Q79R/L99R/E282D	50.0	24.504	0.005
MMLV-II I61M/Q68R/Q79R/L99R/E282D	50.0	24.481	0.009
MMLV-II	51.0	27.277	0.058
MMLV-II Q79R/L99R/E282D	51.0	25.694	0.104
MMLV-II Q68R/Q79R/L99R	51.0	24.579	0.037
MMLV-II Q68R/Q79R/L99R/E282D	51.0	24.364	0.019
MMLV-II Q68R/Q79R/L99K/E282D	51.0	24.849	0.041
MMLV-II Q68R/Q79R/L99N/E282D	51.0	24.899	0.121
MMLV-II Q68K/Q79R/L99R/E282D	51.0	24.980	0.048
MMLV-II Q68R/Q79R/L99R/E282M	51.0	25.292	0.065
MMLV-II I61K/Q68R/Q79R/L99R/E282D	51.0	25.147	0.100
MMLV-II I61M/Q68R/Q79R/L99R/E282D	51.0	25.034	0.075
MMLV-II	51.9	28.797	0.055
MMLV-II Q79R/L99R/E282D	51.9	26.585	0.011
MMLV-II Q68R/Q79R/L99R	51.9	25.021	0.036
MMLV-II Q68R/Q79R/L99R/E282D	51.9	24.763	0.028
MMLV-II Q68R/Q79R/L99K/E282D	51.9	25.392	0.012
MMLV-II Q68R/Q79R/L99N/E282D	51.9	25.543	0.087
MMLV-II Q68K/Q79R/L99R/E282D	51.9	25.549	0.058
MMLV-II Q68R/Q79R/L99R/E282M	51.9	26.025	0.065
MMLV-II I61K/Q68R/Q79R/L99R/E282D	51.9	26.087	0.024
MMLV-II I61M/Q68R/Q79R/L99R/E282D	51.9	25.756	0.054
MMLV-II	53.8	30.985	0.073
MMLV-II Q79R/L99R/E282D	53.8	29.356	0.044
MMLV-II Q68R/Q79R/L99R	53.8	26.370	0.041
MMLV-II Q68R/Q79R/L99R/E282D	53.8	25.580	0.049
MMLV-II Q68R/Q79R/L99K/E282D	53.8	26.682	0.029
MMLV-II Q68R/Q79R/L99N/E282D	53.8	26.438	0.031

MMLV-II Q68K/Q79R/L99R/E282D	53.8	27.024	0.042
MMLV-II Q68R/Q79R/L99R/E282M	53.8	28.314	0.051
MMLV-II I61K/Q68R/Q79R/L99R/E282D	53.8	27.489	0.025
MMLV-II I61M/Q68R/Q79R/L99R/E282D	53.8	27.871	0.118
MMLV-II	56.5	33.313	0.164
MMLV-II Q79R/L99R/E282D	56.5	32.626	0.113
MMLV-II Q68R/Q79R/L99R	56.5	30.047	0.089
MMLV-II Q68R/Q79R/L99R/E282D	56.5	29.183	0.155
MMLV-II Q68R/Q79R/L99K/E282D	56.5	30.750	0.051
MMLV-II Q68R/Q79R/L99N/E282D	56.5	30.403	0.095
MMLV-II Q68K/Q79R/L99R/E282D	56.5	31.707	0.111
MMLV-II Q68R/Q79R/L99R/E282M	56.5	31.878	0.093
MMLV-II I61K/Q68R/Q79R/L99R/E282D	56.5	32.235	0.291
MMLV-II I61M/Q68R/Q79R/L99R/E282D	56.5	32.395	0.105
MMLV-II	59.9	34.408	0.498
MMLV-II Q79R/L99R/E282D	59.9	36.798	2.131
MMLV-II Q68R/Q79R/L99R	59.9	33.997	0.035
MMLV-II Q68R/Q79R/L99R/E282D	59.9	32.009	0.051
MMLV-II Q68R/Q79R/L99K/E282D	59.9	33.685	0.317
MMLV-II Q68R/Q79R/L99N/E282D	59.9	33.083	0.163
MMLV-II Q68K/Q79R/L99R/E282D	59.9	34.160	0.066
MMLV-II Q68R/Q79R/L99R/E282M	59.9	33.650	0.161
MMLV-II I61K/Q68R/Q79R/L99R/E282D	59.9	33.341	0.096
MMLV-II I61M/Q68R/Q79R/L99R/E282D	59.9	34.439	0.222
MMLV-II	62.6	35.163	0.447
MMLV-II Q79R/L99R/E282D	62.6	37.138	1.603
MMLV-II Q68R/Q79R/L99R	62.6	34.108	0.604
MMLV-II Q68R/Q79R/L99R/E282D	62.6	32.539	0.060
MMLV-II Q68R/Q79R/L99K/E282D	62.6	34.175	0.421

MMLV-II Q68R/Q79R/L99N/E282D	62.6	33.726	0.622
MMLV-II Q68K/Q79R/L99R/E282D	62.6	34.376	0.408
MMLV-II Q68R/Q79R/L99R/E282M	62.6	33.792	0.231
MMLV-II I61K/Q68R/Q79R/L99R/E282D	62.6	33.768	0.387
MMLV-II I61M/Q68R/Q79R/L99R/E282D	62.6	34.428	0.085
MMLV-II	64.2	37.284	0.764
MMLV-II Q79R/L99R/E282D	64.2	36.661	0.192
MMLV-II Q68R/Q79R/L99R	64.2	34.463	0.213
MMLV-II Q68R/Q79R/L99R/E282D	64.2	32.992	0.023
MMLV-II Q68R/Q79R/L99K/E282D	64.2	34.805	0.472
MMLV-II Q68R/Q79R/L99N/E282D	64.2	34.060	0.043
MMLV-II Q68K/Q79R/L99R/E282D	64.2	34.508	0.302
MMLV-II Q68R/Q79R/L99R/E282M	64.2	34.481	0.078
MMLV-II I61K/Q68R/Q79R/L99R/E282D	64.2	34.231	0.253
MMLV-II I61M/Q68R/Q79R/L99R/E282D	64.2	35.049	0.885
MMLV-II	65.0	35.809	0.511
MMLV-II Q79R/L99R/E282D	65.0	35.932	0.372
MMLV-II Q68R/Q79R/L99R	65.0	34.979	0.856
MMLV-II Q68R/Q79R/L99R/E282D	65.0	33.293	0.319
MMLV-II Q68R/Q79R/L99K/E282D	65.0	34.974	0.536
MMLV-II Q68R/Q79R/L99N/E282D	65.0	34.862	0.268
MMLV-II Q68K/Q79R/L99R/E282D	65.0	34.363	0.201
MMLV-II Q68R/Q79R/L99R/E282M	65.0	34.687	0.666
MMLV-II I61K/Q68R/Q79R/L99R/E282D	65.0	34.246	0.563
MMLV-II I61M/Q68R/Q79R/L99R/E282D	65.0	34.872	0.467

Example 6: Reverse transcriptase mutant evaluation by oligo dT or random priming

This example demonstrates the procedure used to evaluate each mutant RTase's ability to synthesize cDNA from purified total RNA (DNased, isolated from HeLa cells) compared to the base construct of MMLV RTase. The mutant MMLV RTases were tested by two priming conditions: Oligo dT only and random hexamer priming using a standard two-step cDNA synthesis as described in **Example 5**. The reactions were analyzed and reported by Ct value (**Tables 19 and 20**). Four mutant variants of MMLV RTase showed an increase in the overall activity using oligo dT priming compared to the base construct, Q299E, T332E and V433R. Eight mutant variants of MMLV RTase showed an increase in the overall activity using random priming compared to the base construct, P76R, L82R, I125R, Y271A, L280A, L280R, T328R and V433R.

Table 19 Two-Step cDNA Synthesis by MMLV-RT single mutants using oligo dT priming. The data was generated via qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Ct Mean	Ct Standard Deviation
MMLV-II	40.000	0.000
MMLV-II D209A	40.000	0.000
MMLV-II D209E	40.000	0.000
MMLV-II D209R	40.000	0.000
MMLV-II D83A	40.000	0.000
MMLV-II D83E	40.000	0.000
MMLV-II D83R	40.000	0.000
MMLV-II E201A	40.000	0.000
MMLV-II E201D	40.000	0.000
MMLV-II E201R	40.000	0.000
MMLV-II E367A	40.000	0.000
MMLV-II E367D	40.000	0.000
MMLV-II E367R	40.000	0.000
MMLV-II E596A	40.000	0.000
MMLV-II E596D	40.000	0.000
MMLV-II E596R	40.000	0.000
MMLV-II F210A	40.000	0.000
MMLV-II F210E	40.000	0.000
MMLV-II F210R	40.000	0.000
MMLV-II F369A	40.000	0.000
MMLV-II F369E	40.000	0.000
MMLV-II F369R	40.000	0.000
MMLV-II G308A	40.000	0.000
MMLV-II G308E	40.000	0.000
MMLV-II G308R	40.000	0.000
MMLV-II G331A	40.000	0.000
MMLV-II G331E	40.000	0.000
MMLV-II G331R	40.000	0.000

MMLV-II G73A	40.000	0.000
MMLV-II G73E	40.000	0.000
MMLV-II G73R	40.000	0.000
MMLV-II H77A	40.000	0.000
MMLV-II H77E	40.000	0.000
MMLV-II H77R	40.000	0.000
MMLV-II I125A	40.000	0.000
MMLV-II I125E	40.000	0.000
MMLV-II I125R	40.000	0.000
MMLV-II I212A	40.000	0.000
MMLV-II I212E	40.000	0.000
MMLV-II I212R	40.000	0.000
MMLV-II I593A	40.000	0.000
MMLV-II I593E	40.000	0.000
MMLV-II I593R	40.000	0.000
MMLV-II I597A	40.000	0.000
MMLV-II I597E	40.000	0.000
MMLV-II I597R	40.000	0.000
MMLV-II K285A	40.000	0.000
MMLV-II K285E	40.000	0.000
MMLV-II K285R	40.000	0.000
MMLV-II K348A	40.000	0.000
MMLV-II K348E	40.000	0.000
MMLV-II K348R	40.000	0.000
MMLV-II L198A	40.000	0.000
MMLV-II L198E	40.000	0.000
MMLV-II L198R	40.000	0.000
MMLV-II L280A	40.000	0.000
MMLV-II L280E	40.000	0.000
MMLV-II L280R	40.000	0.000
MMLV-II L352A	40.000	0.000
MMLV-II L352E	40.000	0.000
MMLV-II L352R	40.000	0.000
MMLV-II L357A	40.000	0.000
MMLV-II L357E	40.000	0.000
MMLV-II L357R	40.000	0.000
MMLV-II L82A	40.000	0.000
MMLV-II L82E	40.000	0.000
MMLV-II L82R	40.000	0.000
MMLV-II N335A	39.787	0.302
MMLV-II N335E	40.000	0.000
MMLV-II N335R	40.000	0.000
MMLV-II P76A	40.000	0.000
MMLV-II P76E	40.000	0.000
MMLV-II P76R	40.000	0.000
MMLV-II Q213A	40.000	0.000
MMLV-II Q213E	40.000	0.000
MMLV-II Q213R	40.000	0.000

MMLV-II Q299A	40.000	0.000
MMLV-II Q299E	37.177	3.993
MMLV-II Q299R	40.000	0.000
MMLV-II Q654A	40.000	0.000
MMLV-II Q654E	40.000	0.000
MMLV-II Q654R	40.000	0.000
MMLV-II R205A	40.000	0.000
MMLV-II R205E	39.947	0.075
MMLV-II R205K	40.000	0.000
MMLV-II R211A	40.000	0.000
MMLV-II R211E	40.000	0.000
MMLV-II R211K	40.000	0.000
MMLV-II R311A	40.000	0.000
MMLV-II R311E	40.000	0.000
MMLV-II R311K	40.000	0.000
MMLV-II R389A	40.000	0.000
MMLV-II R389E	40.000	0.000
MMLV-II R389K	40.000	0.000
MMLV-II R650A	40.000	0.000
MMLV-II R650E	40.000	0.000
MMLV-II R650K	40.000	0.000
MMLV-II R657A	40.000	0.000
MMLV-II R657E	39.965	0.050
MMLV-II R657K	40.000	0.000
MMLV-II S67A	40.000	0.000
MMLV-II S67E	40.000	0.000
MMLV-II S67R	36.816	0.703
MMLV-II T328A	40.000	0.000
MMLV-II T328E	40.000	0.000
MMLV-II T328R	40.000	0.000
MMLV-II T332A	39.750	0.354
MMLV-II T332E	38.461	2.177
MMLV-II T332R	40.000	0.000
MMLV-II V129A	40.000	0.000
MMLV-II V129E	40.000	0.000
MMLV-II V129R	40.000	0.000
MMLV-II V433A	40.000	0.000
MMLV-II V433E	40.000	0.000
MMLV-II V433R	38.884	0.806
MMLV-II V476A	40.000	0.000
MMLV-II V476E	40.000	0.000
MMLV-II V476R	40.000	0.000
MMLV-II Y271A	40.000	0.000
MMLV-II Y271E	40.000	0.000
MMLV-II Y271R	40.000	0.000
MMLV-IV	31.467	0.190

Table 20. Two-Step cDNA Synthesis by MMLV-RT single mutants using random priming. The data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Ct Mean	Ct Standard Deviation
MMLV-II	40.000	0.000
MMLV-II D209A	40.000	0.000
MMLV-II D209E	40.000	0.000
MMLV-II D209R	40.000	0.000
MMLV-II D83A	40.000	0.000
MMLV-II D83E	40.000	0.000
MMLV-II D83R	40.000	0.000
MMLV-II E201A	40.000	0.000
MMLV-II E201D	40.000	0.000
MMLV-II E201R	40.000	0.000
MMLV-II E367A	40.000	0.000
MMLV-II E367D	40.000	0.000
MMLV-II E367R	40.000	0.000
MMLV-II E596A	40.000	0.000
MMLV-II E596D	40.000	0.000
MMLV-II E596R	40.000	0.000
MMLV-II F210A	40.000	0.000
MMLV-II F210E	40.000	0.000
MMLV-II F210R	40.000	0.000
MMLV-II F369A	40.000	0.000
MMLV-II F369E	40.000	0.000
MMLV-II F369R	40.000	0.000
MMLV-II G308A	40.000	0.000
MMLV-II G308E	40.000	0.000
MMLV-II G308R	40.000	0.000
MMLV-II G331A	40.000	0.000
MMLV-II G331E	40.000	0.000
MMLV-II G331R	40.000	0.000
MMLV-II G73A	40.000	0.000
MMLV-II G73E	40.000	0.000
MMLV-II G73R	40.000	0.000
MMLV-II H77A	39.708	0.412
MMLV-II H77E	40.000	0.000
MMLV-II H77R	40.000	0.000
MMLV-II I125A	40.000	0.000
MMLV-II I125E	40.000	0.000
MMLV-II I125R	39.449	0.779
MMLV-II I212A	40.000	0.000
MMLV-II I212E	40.000	0.000
MMLV-II I212R	40.000	0.000
MMLV-II I593A	40.000	0.000
MMLV-II I593E	40.000	0.000
MMLV-II I593R	40.000	0.000
MMLV-II I597A	40.000	0.000

MMLV-II I597E	40.000	0.000
MMLV-II I597R	40.000	0.000
MMLV-II K285A	40.000	0.000
MMLV-II K285E	40.000	0.000
MMLV-II K285R	39.783	0.308
MMLV-II K348A	40.000	0.000
MMLV-II K348E	40.000	0.000
MMLV-II K348R	40.000	0.000
MMLV-II L198A	40.000	0.000
MMLV-II L198E	40.000	0.000
MMLV-II L198R	40.000	0.000
MMLV-II L280A	39.503	0.703
MMLV-II L280E	40.000	0.000
MMLV-II L280R	38.762	1.751
MMLV-II L352A	39.778	0.313
MMLV-II L352E	40.000	0.000
MMLV-II L352R	40.000	0.000
MMLV-II L357A	40.000	0.000
MMLV-II L357E	40.000	0.000
MMLV-II L357R	40.000	0.000
MMLV-II L82A	40.000	0.000
MMLV-II L82E	39.673	0.462
MMLV-II L82R	38.926	1.518
MMLV-II N335A	39.876	0.175
MMLV-II N335E	40.000	0.000
MMLV-II N335R	39.861	0.196
MMLV-II P76A	40.000	0.000
MMLV-II P76E	40.000	0.000
MMLV-II P76R	39.535	0.658
MMLV-II Q213A	40.000	0.000
MMLV-II Q213E	40.000	0.000
MMLV-II Q213R	40.000	0.000
MMLV-II Q299A	40.000	0.000
MMLV-II Q299E	40.000	0.000
MMLV-II Q299R	40.000	0.000
MMLV-II Q654A	40.000	0.000
MMLV-II Q654E	40.000	0.000
MMLV-II Q654R	40.000	0.000
MMLV-II R205A	39.811	0.267
MMLV-II R205E	40.000	0.000
MMLV-II R205K	40.000	0.000
MMLV-II R211A	40.000	0.000
MMLV-II R211E	40.000	0.000
MMLV-II R211K	40.000	0.000
MMLV-II R311A	40.000	0.000
MMLV-II R311E	40.000	0.000
MMLV-II R311K	40.000	0.000
MMLV-II R389A	40.000	0.000

MMLV-II R389E	40.000	0.000
MMLV-II R389K	40.000	0.000
MMLV-II R650A	40.000	0.000
MMLV-II R650E	40.000	0.000
MMLV-II R650K	40.000	0.000
MMLV-II R657A	40.000	0.000
MMLV-II R657E	40.000	0.000
MMLV-II R657K	40.000	0.000
MMLV-II S67A	40.000	0.000
MMLV-II S67E	39.435	0.800
MMLV-II S67R	38.209	0.977
MMLV-II T328A	40.000	0.000
MMLV-II T328E	40.000	0.000
MMLV-II T328R	39.478	0.739
MMLV-II T332A	40.000	0.000
MMLV-II T332E	40.000	0.000
MMLV-II T332R	40.000	0.000
MMLV-II V129A	40.000	0.000
MMLV-II V129E	40.000	0.000
MMLV-II V129R	40.000	0.000
MMLV-II V433A	40.000	0.000
MMLV-II V433E	40.000	0.000
MMLV-II V433R	38.071	1.452
MMLV-II V476A	40.000	0.000
MMLV-II V476E	40.000	0.000
MMLV-II V476R	40.000	0.000
MMLV-II Y271A	39.466	0.755
MMLV-II Y271E	40.000	0.000
MMLV-II Y271R	40.000	0.000
MMLV-IV	31.850	0.183

Example 7. Reverse transcriptase mutant evaluation by gene specific priming

This example demonstrates the procedure used to evaluate each mutant RTase's ability to synthesize cDNA from purified RNA ultramers (Integrated DNA Technologies) compared to the base construct of MMLV RTase. The mutant MMLV RTases were tested by a one-step addition of the RTase in GEM as described in **Example 5**. The reactions were analyzed and reported by Ct value (**Table 21**). Twelve mutant variants of MMLV RTase showed an increase in the overall activity compared to the base construct, H77A, D83E, D83R, Y271E, Q299E, G308E, F396A, V433R, I593E, I597A and I597R.

Table 21 One-Step cDNA Synthesis by MMLV-RT single mutants by gene specific priming. The data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Ct Mean	Ct Standard Deviation
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MMLV-II	29.065	0.277
MMLV-II D209A	29.583	0.166
MMLV-II D209E	28.900	0.088
MMLV-II D209R	29.266	0.068
MMLV-II D83A	29.588	0.082
MMLV-II D83E	28.499	0.087
MMLV-II D83R	28.724	0.087
MMLV-II E201A	30.692	0.173
MMLV-II E201D	29.130	0.157
MMLV-II E201R	29.333	0.141
MMLV-II E367A	31.153	0.021
MMLV-II E367D	31.070	0.187
MMLV-II E367R	34.221	0.475
MMLV-II E596A	29.150	0.121
MMLV-II E596D	30.494	0.081
MMLV-II E596R	31.787	0.227
MMLV-II F210A	33.639	0.196
MMLV-II F210E	34.982	0.065
MMLV-II F210R	37.201	1.986
MMLV-II F369A	29.055	0.063
MMLV-II F369E	36.856	0.508
MMLV-II F369R	36.149	0.308
MMLV-II G308A	30.226	0.170
MMLV-II G308E	28.772	0.121
MMLV-II G308R	40.000	0.000
MMLV-II G331A	30.412	0.137
MMLV-II G331E	31.321	0.160
MMLV-II G331R	31.340	0.020
MMLV-II G73A	30.741	0.125
MMLV-II G73E	34.319	0.369
MMLV-II G73R	29.721	0.061
MMLV-II H77A	28.581	0.070
MMLV-II H77E	29.475	0.107
MMLV-II H77R	29.726	0.120
MMLV-II I125A	29.812	0.043
MMLV-II I125E	30.712	0.147
MMLV-II I125R	30.324	0.012
MMLV-II I212A	29.586	0.086
MMLV-II I212E	29.459	0.073
MMLV-II I212R	29.037	0.092
MMLV-II I593A	30.560	0.101
MMLV-II I593E	27.779	0.056
MMLV-II I593R	29.268	0.012
MMLV-II I597A	28.983	0.024
MMLV-II I597E	29.583	0.143
MMLV-II I597R	28.671	0.103
MMLV-II K285A	32.375	0.158
MMLV-II K285E	37.065	0.044

MMLV-II K285R	30.564	0.075
MMLV-II K348A	34.241	0.516
MMLV-II K348E	34.533	0.432
MMLV-II K348R	29.703	0.225
MMLV-II L198A	31.900	0.054
MMLV-II L198E	34.193	0.167
MMLV-II L198R	30.819	0.077
MMLV-II L280A	35.724	0.175
MMLV-II L280E	40.000	0.000
MMLV-II L280R	40.000	0.000
MMLV-II L352A	28.936	0.043
MMLV-II L352E	30.177	0.059
MMLV-II L352R	29.371	0.063
MMLV-II L357A	38.802	1.694
MMLV-II L357E	40.000	0.000
MMLV-II L357R	40.000	0.000
MMLV-II L82A	31.245	0.035
MMLV-II L82E	31.384	0.122
MMLV-II L82R	29.682	0.116
MMLV-II N335A	29.668	0.086
MMLV-II N335E	29.113	0.058
MMLV-II N335R	32.323	5.429
MMLV-II P76A	29.463	0.123
MMLV-II P76E	30.030	0.163
MMLV-II P76R	29.443	0.028
MMLV-II Q213A	29.833	0.223
MMLV-II Q213E	29.677	0.196
MMLV-II Q213R	29.704	0.053
MMLV-II Q299A	31.314	0.200
MMLV-II Q299E	28.652	0.149
MMLV-II Q299R	31.711	0.062
MMLV-II Q654A	29.415	0.117
MMLV-II Q654E	30.523	0.057
MMLV-II Q654R	29.523	0.052
MMLV-II R205A	29.140	0.138
MMLV-II R205E	29.356	0.179
MMLV-II R205K	29.162	0.206
MMLV-II R211A	29.491	0.025
MMLV-II R211E	30.049	0.205
MMLV-II R211K	30.196	0.147
MMLV-II R311A	31.237	0.425
MMLV-II R311E	40.000	0.000
MMLV-II R311K	29.857	0.091
MMLV-II R389A	32.173	0.151
MMLV-II R389E	32.717	0.105
MMLV-II R389K	31.944	0.166
MMLV-II R650A	29.734	0.060
MMLV-II R650E	31.012	0.074

MMLV-II R650K	29.404	0.094
MMLV-II R657A	31.470	0.133
MMLV-II R657E	32.785	0.145
MMLV-II R657K	29.468	0.274
MMLV-II S67A	29.268	0.090
MMLV-II S67E	30.157	0.254
MMLV-II S67R	27.274	0.054
MMLV-II T328A	40.000	0.000
MMLV-II T328E	37.699	1.627
MMLV-II T328R	37.169	0.848
MMLV-II T332A	29.219	0.075
MMLV-II T332E	29.714	0.057
MMLV-II T332R	30.462	0.130
MMLV-II V129A	29.305	0.077
MMLV-II V129E	31.188	0.181
MMLV-II V129R	30.383	0.081
MMLV-II V433A	30.483	0.059
MMLV-II V433E	30.106	0.144
MMLV-II V433R	29.297	0.457
MMLV-II V476A	31.295	0.244
MMLV-II V476E	34.664	0.364
MMLV-II V476R	31.223	0.166
MMLV-II Y271A	30.854	0.086
MMLV-II Y271E	28.620	0.068
MMLV-II Y271R	33.280	0.258
MMLV-IV	26.368	0.057

Example 8. Further stacking of reverse transcriptase mutants with enhanced activity.

This example demonstrates the procedure used to stack the enhanced mutants found in **Examples 6-7** to further improve the MMLV RTase's ability to synthesize cDNA from purified total RNA (DNased, isolated from HeLa cells) compared to the the base construct and previously found mutant MMLV RTase containing the following mutations: Q68R/Q79R/L99R/E282D. The stacked mutant MMLV RTases were cloned, overexpressed and purified as described in **Examples 1 – 2** and tested as described in **Examples 6-7**. Both the two- and one-step reactions were analyzed and reported by Ct value (**Table 22-24**). Six of the eight stacked mutant variants of MMLV RTase increased the overall activity and thermostability compared to the base construct, Q68R/Q79R/L99R/E282D/V433R, Q68R/Q79R/L99R/E282D/I593E, Q68R/Q79R/L99R/E282D/Q299E, Q68R/Q79R/L99R/E282D/T332E, Q68R/L82R/L99R/E282D and Q68R/Q79R/L82R/L99R/E282D. Subsequently, four of those six stacked mutant variants of MMLV RTase increased the overall activity and thermostability compared to the previously identified mutant RTase (Q68R/Q79R/L99R/E282D),

Q68R/Q79R/L99R/E282D/I593E, Q68R/Q79R/L99R/E282D/Q299E, Q68R/L82R/L99R/E282D and Q68R/Q79R/L82R/L99R/E282D.

Following these stacked mutant variants, MMLV RTase mutations were stacked further to improve the ability of MMLV RTase to synthesize cDNA from purified total RNA (DNased, isolated from HeLa cells) as compared to the MMLV RTase base construct (RNase H minus construct). Eight MMLV RTase sextuple or more mutant variants were cloned as described in **Example 1** and overexpressed and purified as in **Example 5**.

MMLV RTase base construct and MMLV RTase mutant variants evaluated as described in Example 3. Temperatures were adjusted for both two-step and one-step reactions to 42/55 and 50/60°C, respectively. The two-step first strand synthesis buffer was modified from 50 mM Tris-hydrochloride, pH 8.3, 75 mM potassium chloride, 3 mM magnesium chloride and 10 mM DTT to 50 mM potassium acetate, 20 mM Tris-acetate, pH 7.0, 10 mM magnesium acetate, 100 µg/ml bovine serum albumin and 10 mM DTT. The two-step and one-step reactions for MMLV RTase base construct and MMLV RTase mutant variants were analyzed and reported by Ct output from the qPCR (**Tables 22-24**).

Four of the eleven MMLV RTase sextuple or more mutant variants were found to exhibit increased overall activity and thermostability as compared to the other MMLV RTase stacked mutant variants, and almost all of the MMLV RTase stacked mutant variants exhibited increased overall activity and thermostability as compared to the MMLV RTase base construct. The four MMLV RTase mutant variants that were found to exhibit the highest overall activity were Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E, Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E, Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E and Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E.

Table 22. Two-Step cDNA Synthesis by MMLV-RT stacked mutants using oligo dT priming. The data was generated via qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Ct Mean	Ct Standard Deviation
MMLV-II	37.388	0.396
MMLV-II Q68R/Q79R/L99R/E282D/V433R	29.215	0.113
MMLV-II Q68R/Q79R/L99R/E282D/I593E	33.563	0.118
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	31.902	0.169
MMLV-II Q68R/Q79R/L99R/E282D/T332E	33.988	0.108
MMLV-II Q68R/Q79R/L99R/L280R	40.000	0.000
MMLV-II Q68R/Q79R/L99R/L280R/E282D	40.000	0.000
MMLV-II Q68R/L82R/L99R/E282D	39.259	1.047

MMLV-II Q68R/Q79R/L82R/L99R/E282D	30.623	0.076
MMLV-IV	25.880	0.023

Table 23. Two-Step cDNA Synthesis by MMLV-RT stacked mutants using random priming. The data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Ct Mean	Ct Standard Deviation
MMLV-II	36.638	1.014
MMLV-II Q68R/Q79R/L99R/E282D/V433R	40.000	0.000
MMLV-II Q68R/Q79R/L99R/E282D/I593E	32.331	0.111
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	30.430	0.154
MMLV-II Q68R/Q79R/L99R/E282D/T332E	33.720	0.266
MMLV-II Q68R/Q79R/L99R/L280R	40.000	0.000
MMLV-II Q68R/Q79R/L99R/L280R/E282D	40.000	0.000
MMLV-II Q68R/L82R/L99R/E282D	35.325	0.422
MMLV-II Q68R/Q79R/L82R/L99R/E282D	31.928	0.177
MMLV-IV	25.840	0.049

Table 24. One-Step cDNA Synthesis by MMLV-RT stacked mutants by gene specific priming. The data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Ct Mean	Ct Standard Deviation
MMLV-II	33.027	0.048
MMLV-II Q68R/Q79R/L99R/E282D/V433R	29.937	0.040
MMLV-II Q68R/Q79R/L99R/E282D/I593E	28.724	0.081
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	29.341	0.022
MMLV-II Q68R/Q79R/L99R/E282D/T332E	30.330	0.036
MMLV-II Q68R/Q79R/L99R/L280R	40.000	0.000
MMLV-II Q68R/Q79R/L99R/L280R/E282D	40.000	0.000
MMLV-II Q68R/L82R/L99R/E282D	30.559	0.045
MMLV-II Q68R/Q79R/L82R/L99R/E282D	30.097	0.033
MMLV-IV	28.975	0.012

a. Evaluation of ability of purified MMLV RTase mutant variants to synthesize DNA over a wide range of temperatures

MMLV RTase base construct MMLV RTase mutant variants evaluated as described in Example 5. Oligo-dT or random hexamer priming conditions and reaction temperatures were adjusted for the two-step reactions and RTase concentration was normalized to 31 nM. The two-step reactions for MMLV RTase base construct and MMLV RTase mutant variants were analyzed and reported by Ct output from the qPCR (see tables 25 and 26)

Five MMLV RTase mutants were found to exhibit high overall activity as compared to the MMLV RTase base construct over a wide range of temperatures, spanning from 37.0 to 51 °C, regardless of which priming method used. All of the MMLV RTase stacked mutant

variants exhibited increased overall activity and thermostability as compared to the MMLV RTase base construct. The five MMLV RTas mutant variants that were found to exhibit the highest overall activity at a wide range of temperatures were Q68R/Q79R/L99R/E282D, Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E, Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E, Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E and Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E

Table 25. Two-Step cDNA synthesis by MMLV RT quadruple and more mutants by Oligo-dT priming. Data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV RT Mutant	Temperature of Reaction (°C)	Ct Mean	Ct SD
MMLV-II	37.0	26.340	0.033
MMLV-II	37.8	26.130	0.061
MMLV-II	39.5	25.830	0.014
MMLV-II	42.0	25.753	0.041
MMLV-II	45.2	25.632	0.077
MMLV-II	47.8	25.935	0.026
MMLV-II	49.2	26.478	0.042
MMLV-II	50.0	29.461	0.120
MMLV-II	51.0	29.430	0.098
MMLV-II	51.9	31.123	0.066
MMLV-II	53.8	33.632	0.073
MMLV-II	56.5	36.499	0.385
MMLV-II	59.9	37.158	0.427
MMLV-II	62.6	37.464	0.440
MMLV-II	64.2	37.082	0.022
MMLV-II	65.0	37.518	0.370
MMLV-II Q68R/Q79R/L99R/E282D	37.0	25.688	0.031
MMLV-II Q68R/Q79R/L99R/E282D	37.8	25.734	0.032
MMLV-II Q68R/Q79R/L99R/E282D	39.5	25.613	0.040
MMLV-II Q68R/Q79R/L99R/E282D	42.0	25.528	0.032
MMLV-II Q68R/Q79R/L99R/E282D	45.2	25.525	0.029
MMLV-II Q68R/Q79R/L99R/E282D	47.8	25.471	0.105
MMLV-II Q68R/Q79R/L99R/E282D	49.2	25.491	0.047
MMLV-II Q68R/Q79R/L99R/E282D	50.0	25.608	0.061
MMLV-II Q68R/Q79R/L99R/E282D	51.0	25.679	0.006
MMLV-II Q68R/Q79R/L99R/E282D	51.9	25.969	0.032
MMLV-II Q68R/Q79R/L99R/E282D	53.8	27.251	0.053
MMLV-II Q68R/Q79R/L99R/E282D	56.5	33.619	0.195

MMLV-II Q68R/Q79R/L99R/E282D	59.9	36.635	0.059
MMLV-II Q68R/Q79R/L99R/E282D	62.6	36.929	0.500
MMLV-II Q68R/Q79R/L99R/E282D	64.2	37.515	0.478
MMLV-II Q68R/Q79R/L99R/E282D	65.0	37.107	0.285
MMLV-II Q68R/Q79R/L99R/E282D/I593E	37.0	26.133	0.054
MMLV-II Q68R/Q79R/L99R/E282D/I593E	37.8	26.029	0.012
MMLV-II Q68R/Q79R/L99R/E282D/I593E	39.5	25.850	0.047
MMLV-II Q68R/Q79R/L99R/E282D/I593E	42.0	25.793	0.012
MMLV-II Q68R/Q79R/L99R/E282D/I593E	45.2	25.614	0.018
MMLV-II Q68R/Q79R/L99R/E282D/I593E	47.8	25.658	0.005
MMLV-II Q68R/Q79R/L99R/E282D/I593E	49.2	25.663	0.024
MMLV-II Q68R/Q79R/L99R/E282D/I593E	50.0	25.791	0.041
MMLV-II Q68R/Q79R/L99R/E282D/I593E	51.0	25.877	0.067
MMLV-II Q68R/Q79R/L99R/E282D/I593E	51.9	26.602	0.038
MMLV-II Q68R/Q79R/L99R/E282D/I593E	53.8	29.535	0.086
MMLV-II Q68R/Q79R/L99R/E282D/I593E	56.5	35.912	0.439
MMLV-II Q68R/Q79R/L99R/E282D/I593E	59.9	37.158	0.566
MMLV-II Q68R/Q79R/L99R/E282D/I593E	62.6	37.187	0.158
MMLV-II Q68R/Q79R/L99R/E282D/I593E	64.2	37.958	0.236
MMLV-II Q68R/Q79R/L99R/E282D/I593E	65.0	36.861	0.416
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	37.0	26.106	0.070
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	37.8	26.024	0.092
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	39.5	25.830	0.122
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	42.0	25.788	0.025
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	45.2	25.634	0.022
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	47.8	25.681	0.016
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	49.2	25.684	0.029
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	50.0	25.743	0.096
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	51.0	25.870	0.003
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	51.9	26.301	0.033
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	53.8	28.283	0.036
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	56.5	34.732	0.445
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	59.9	36.947	0.407
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	62.6	37.140	0.280
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	64.2	37.403	0.205
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	65.0	37.347	0.438
MMLV-II Q68R/Q79R/L82R/L99R/E282D	37.0	25.961	0.170
MMLV-II Q68R/Q79R/L82R/L99R/E282D	37.8	26.065	0.085
MMLV-II Q68R/Q79R/L82R/L99R/E282D	39.5	25.909	0.028
MMLV-II Q68R/Q79R/L82R/L99R/E282D	42.0	25.802	0.055
MMLV-II Q68R/Q79R/L82R/L99R/E282D	45.2	25.632	0.087
MMLV-II Q68R/Q79R/L82R/L99R/E282D	47.8	25.728	0.065
MMLV-II Q68R/Q79R/L82R/L99R/E282D	49.2	25.612	0.165
MMLV-II Q68R/Q79R/L82R/L99R/E282D	50.0	25.795	0.038
MMLV-II Q68R/Q79R/L82R/L99R/E282D	51.0	25.830	0.009

MMLV-II Q68R/Q79R/L82R/L99R/E282D	51.9	26.477	0.037
MMLV-II Q68R/Q79R/L82R/L99R/E282D	53.8	28.496	0.040
MMLV-II Q68R/Q79R/L82R/L99R/E282D	56.5	34.329	0.177
MMLV-II Q68R/Q79R/L82R/L99R/E282D	59.9	36.564	0.315
MMLV-II Q68R/Q79R/L82R/L99R/E282D	62.6	37.152	0.322
MMLV-II Q68R/Q79R/L82R/L99R/E282D	64.2	37.340	0.585
MMLV-II Q68R/Q79R/L82R/L99R/E282D	65.0	38.351	1.016
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	37.0	25.853	0.057
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	37.8	25.898	0.016
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	39.5	25.716	0.093
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	42.0	25.669	0.064
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	45.2	25.643	0.056
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	47.8	25.680	0.016
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	49.2	25.663	0.057
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	50.0	25.708	0.045
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	51.0	25.557	0.025
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	51.9	26.015	0.125
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	53.8	27.812	0.048
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	56.5	34.073	0.217
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	59.9	36.512	0.168
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	62.6	37.182	0.167
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	64.2	37.239	0.291
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	65.0	36.573	0.232
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	37.0	25.789	0.075
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	37.8	25.784	0.103
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	39.5	25.714	0.025
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	42.0	25.713	0.027
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	45.2	25.690	0.030
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	47.8	25.662	0.026
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	49.2	25.713	0.021
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	50.0	25.551	0.092
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	51.0	25.561	0.107
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	51.9	25.975	0.125
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	53.8	27.556	0.023
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	56.5	33.934	0.249

MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	59.9	36.473	0.285
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	62.6	37.411	0.377
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	64.2	37.656	0.478
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	65.0	37.950	1.451
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	37.0	25.788	0.028
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	37.8	25.680	0.229
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	39.5	25.794	0.051
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	42.0	25.415	0.270
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	45.2	25.631	0.047
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	47.8	25.672	0.027
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	49.2	25.792	0.045
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	50.0	25.759	0.022
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	51.0	25.852	0.015
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	51.9	26.425	0.033
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	53.8	29.964	0.023
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	56.5	36.532	0.113
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	59.9	38.246	0.608
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	62.6	37.333	0.446
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	64.2	37.223	0.212
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	65.0	36.930	0.527
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	37.0	25.863	0.014
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	37.8	25.649	0.036
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	39.5	25.573	0.057
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	42.0	25.453	0.023
MML V-II	45.2	25.447	0.083

Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E			
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	47.8	25.413	0.061
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	49.2	25.542	0.035
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	50.0	25.567	0.060
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	51.0	25.741	0.093
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	51.9	26.231	0.225
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	53.8	28.556	0.142
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	56.5	35.202	0.208
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	59.9	36.991	0.419
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	62.6	37.168	0.463
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	64.2	37.670	0.410
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	65.0	37.680	0.273

Table 26. Two-Step cDNA synthesis by MMLV RT quadruple and more mutants by Random priming. Data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV RT Mutant	Temperature of Reaction (°C)	Ct Mean	Ct SD
MMLV-II	37.0	26.365	0.066
MMLV-II	37.8	26.390	0.006
MMLV-II	39.5	25.939	0.016
MMLV-II	42.0	25.798	0.029
MMLV-II	45.2	25.849	0.064
MMLV-II	47.8	26.647	0.050
MMLV-II	49.2	28.326	0.028
MMLV-II	50.0	29.340	0.010
MMLV-II	51.0	30.684	0.099
MMLV-II	51.9	32.462	0.163
MMLV-II	53.8	33.855	0.307
MMLV-II	56.5	35.376	0.461
MMLV-II	59.9	36.098	0.481
MMLV-II	62.6	36.391	0.367
MMLV-II	64.2	36.442	0.547
MMLV-II	65.0	35.871	0.301
MMLV-II Q68R/Q79R/L99R/E282D	37.0	25.699	0.009

MMLV-II Q68R/Q79R/L99R/E282D	37.8	25.674	0.038
MMLV-II Q68R/Q79R/L99R/E282D	39.5	25.594	0.029
MMLV-II Q68R/Q79R/L99R/E282D	42.0	25.496	0.016
MMLV-II Q68R/Q79R/L99R/E282D	45.2	25.431	0.011
MMLV-II Q68R/Q79R/L99R/E282D	47.8	25.420	0.036
MMLV-II Q68R/Q79R/L99R/E282D	49.2	25.481	0.023
MMLV-II Q68R/Q79R/L99R/E282D	50.0	25.646	0.035
MMLV-II Q68R/Q79R/L99R/E282D	51.0	25.979	0.012
MMLV-II Q68R/Q79R/L99R/E282D	51.9	26.591	0.053
MMLV-II Q68R/Q79R/L99R/E282D	53.8	28.345	0.091
MMLV-II Q68R/Q79R/L99R/E282D	56.5	32.976	0.109
MMLV-II Q68R/Q79R/L99R/E282D	59.9	34.407	0.158
MMLV-II Q68R/Q79R/L99R/E282D	62.6	35.130	0.014
MMLV-II Q68R/Q79R/L99R/E282D	64.2	34.866	0.258
MMLV-II Q68R/Q79R/L99R/E282D	65.0	35.317	0.299
MMLV-II Q68R/Q79R/L99R/E282D/I593E	37.0	26.079	0.036
MMLV-II Q68R/Q79R/L99R/E282D/I593E	37.8	25.951	0.015
MMLV-II Q68R/Q79R/L99R/E282D/I593E	39.5	25.801	0.055
MMLV-II Q68R/Q79R/L99R/E282D/I593E	42.0	25.602	0.087
MMLV-II Q68R/Q79R/L99R/E282D/I593E	45.2	25.424	0.038
MMLV-II Q68R/Q79R/L99R/E282D/I593E	47.8	25.520	0.011
MMLV-II Q68R/Q79R/L99R/E282D/I593E	49.2	25.674	0.046
MMLV-II Q68R/Q79R/L99R/E282D/I593E	50.0	25.922	0.015
MMLV-II Q68R/Q79R/L99R/E282D/I593E	51.0	26.351	0.014
MMLV-II Q68R/Q79R/L99R/E282D/I593E	51.9	27.411	0.092
MMLV-II Q68R/Q79R/L99R/E282D/I593E	53.8	30.482	0.048
MMLV-II Q68R/Q79R/L99R/E282D/I593E	56.5	33.914	0.075
MMLV-II Q68R/Q79R/L99R/E282D/I593E	59.9	35.443	0.191
MMLV-II Q68R/Q79R/L99R/E282D/I593E	62.6	35.872	0.445
MMLV-II Q68R/Q79R/L99R/E282D/I593E	64.2	36.107	0.011
MMLV-II Q68R/Q79R/L99R/E282D/I593E	65.0	35.715	0.299
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	37.0	25.955	0.040
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	37.8	25.934	0.023
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	39.5	25.669	0.035
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	42.0	25.523	0.016
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	45.2	25.532	0.054
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	47.8	25.550	0.021
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	49.2	25.620	0.030
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	50.0	25.711	0.035
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	51.0	26.215	0.056
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	51.9	26.969	0.013
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	53.8	29.622	0.060
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	56.5	33.679	0.234
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	59.9	35.253	0.144
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	62.6	35.408	0.441

MMLV-II Q68R/Q79R/L99R/E282D/Q299E	64.2	35.586	0.139
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	65.0	36.076	0.700
MMLV-II Q68R/Q79R/L82R/L99R/E282D	37.0	25.884	0.012
MMLV-II Q68R/Q79R/L82R/L99R/E282D	37.8	25.833	0.009
MMLV-II Q68R/Q79R/L82R/L99R/E282D	39.5	25.684	0.077
MMLV-II Q68R/Q79R/L82R/L99R/E282D	42.0	25.553	0.026
MMLV-II Q68R/Q79R/L82R/L99R/E282D	45.2	25.471	0.043
MMLV-II Q68R/Q79R/L82R/L99R/E282D	47.8	25.491	0.085
MMLV-II Q68R/Q79R/L82R/L99R/E282D	49.2	25.646	0.014
MMLV-II Q68R/Q79R/L82R/L99R/E282D	50.0	25.765	0.039
MMLV-II Q68R/Q79R/L82R/L99R/E282D	51.0	26.365	0.044
MMLV-II Q68R/Q79R/L82R/L99R/E282D	51.9	27.170	0.071
MMLV-II Q68R/Q79R/L82R/L99R/E282D	53.8	29.662	0.048
MMLV-II Q68R/Q79R/L82R/L99R/E282D	56.5	33.853	0.162
MMLV-II Q68R/Q79R/L82R/L99R/E282D	59.9	34.899	0.325
MMLV-II Q68R/Q79R/L82R/L99R/E282D	62.6	35.557	0.145
MMLV-II Q68R/Q79R/L82R/L99R/E282D	64.2	35.360	0.222
MMLV-II Q68R/Q79R/L82R/L99R/E282D	65.0	35.614	0.403
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	37.0	25.706	0.031
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	37.8	25.757	0.101
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	39.5	25.435	0.036
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	42.0	25.417	0.025
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	45.2	25.425	0.023
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	47.8	25.401	0.049
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	49.2	25.467	0.009
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	50.0	25.516	0.056
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	51.0	25.880	0.039
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	51.9	26.348	0.064
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	53.8	28.506	0.018
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	56.5	32.812	0.242
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	59.9	34.123	0.163
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	62.6	35.108	0.027
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	64.2	34.796	0.171
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	65.0	34.999	0.064
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	37.0	25.711	0.080
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	37.8	25.916	0.224
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	39.5	25.665	0.052
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	42.0	25.527	0.016
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	45.2	25.504	0.065
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	47.8	25.437	0.070

MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	49.2	25.555	0.065
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	50.0	25.571	0.028
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	51.0	25.854	0.029
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	51.9	26.259	0.057
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	53.8	28.329	0.053
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	56.5	32.962	0.212
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	59.9	34.072	0.446
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	62.6	34.931	0.205
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	64.2	34.626	0.169
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	65.0	35.085	0.230
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	37.0	25.940	0.130
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	37.8	25.793	0.129
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	39.5	25.599	0.015
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	42.0	25.504	0.016
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	45.2	25.602	0.041
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	47.8	25.604	0.058
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	49.2	25.665	0.007
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	50.0	25.821	0.068
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	51.0	26.315	0.047
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	51.9	27.036	0.059
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	53.8	31.004	0.089
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	56.5	33.765	0.274
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	59.9	34.656	0.209
MML V-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	62.6	35.561	0.468
MML V-II	64.2	35.877	0.154

Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E			
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	65.0	35.659	0.477
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	37.0	25.780	0.046
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	37.8	25.652	0.026
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	39.5	25.641	0.037
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	42.0	25.507	0.005
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	45.2	25.484	0.067
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	47.8	25.438	0.027
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	49.2	25.534	0.022
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	50.0	25.755	0.085
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	51.0	25.981	0.027
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	51.9	26.242	0.052
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	53.8	29.146	0.069
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	56.5	33.138	0.159
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	59.9	34.551	0.152
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	62.6	35.186	0.322
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	64.2	35.550	0.368
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	65.0	35.459	0.295

Example 9: Extension of Reverse Transcriptase Single Mutants

The amino acid positions that enclosed the MMLV RTase single mutants identified in **Examples 6 and 7** were further evaluated to include all possible amino acid substitutions at that position. The single mutants were cloned, overexpressed, and purified as described in **Examples 1 and 2**, and evaluated as described in **Examples 6 and 7**. The two-step and one-step reactions for MMLV RTase base construct and MMLV RTase double mutant variants were analyzed and reported by Ct output from the qPCR (**Tables 27-29**). Numerous single mutant MMLV RTase variants were found to exhibit an increase in the overall activity and

thermostability as compared to the MMLV RTase base construct. The most prevalent among these were: L82F, L82K, L82T, L82Y, L280I, T332V, V433K, V433N and I593W.

Table 27. Two-Step cDNA Synthesis by MMLV-RT single mutants using Oligo-dT priming. The data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Ct Mean	Ct Standard Deviation
MMLV-II	40.000	0.000
MMLV-II I593A	40.000	0.000
MMLV-II I593C	37.874	0.991
MMLV-II I593D	40.000	0.000
MMLV-II I593E	40.000	0.000
MMLV-II I593F	40.000	0.000
MMLV-II I593G	39.748	0.356
MMLV-II I593H	39.502	0.704
MMLV-II I593K	40.000	0.000
MMLV-II I593L	38.994	1.423
MMLV-II I593M	39.383	0.873
MMLV-II I593N	40.000	0.000
MMLV-II I593P	40.000	0.000
MMLV-II I593Q	40.000	0.000
MMLV-II I593R	40.000	0.000
MMLV-II I593S	39.614	0.545
MMLV-II I593T	37.709	0.520
MMLV-II I593V	40.000	0.000
MMLV-II I593W	30.504	0.073
MMLV-II I593Y	40.000	0.000
MMLV-II L280A	40.000	0.000
MMLV-II L280C	40.000	0.000
MMLV-II L280D	40.000	0.000
MMLV-II L280E	40.000	0.000
MMLV-II L280F	40.000	0.000
MMLV-II L280G	40.000	0.000
MMLV-II L280H	40.000	0.000
MMLV-II L280I	30.951	0.076
MMLV-II L280K	40.000	0.000
MMLV-II L280M	40.000	0.000
MMLV-II L280N	39.727	0.386
MMLV-II L280P	40.000	0.000
MMLV-II L280Q	40.000	0.000
MMLV-II L280R	39.994	0.009
MMLV-II L280S	40.000	0.000
MMLV-II L280T	40.000	0.000
MMLV-II L280V	37.749	0.142

MMLV-II L280W	40.000	0.000
MMLV-II L280Y	40.000	0.000
MMLV-II L82A	40.000	0.000
MMLV-II L82C	39.565	0.615
MMLV-II L82D	40.000	0.000
MMLV-II L82E	40.000	0.000
MMLV-II L82F	39.347	0.924
MMLV-II L82G	40.000	0.000
MMLV-II L82H	40.000	0.000
MMLV-II L82I	40.000	0.000
MMLV-II L82K	37.136	0.593
MMLV-II L82M	38.649	1.260
MMLV-II L82N	40.000	0.000
MMLV-II L82P	40.000	0.000
MMLV-II L82Q	39.098	1.275
MMLV-II L82R	40.000	0.000
MMLV-II L82S	39.346	0.925
MMLV-II L82T	38.695	1.845
MMLV-II L82V	38.047	1.381
MMLV-II L82W	37.151	0.308
MMLV-II L82Y	35.014	0.421
MMLV-II Q299A	40.000	0.000
MMLV-II Q299C	40.000	0.000
MMLV-II Q299D	40.000	0.000
MMLV-II Q299E	39.061	1.328
MMLV-II Q299F	40.000	0.000
MMLV-II Q299G	40.000	0.000
MMLV-II Q299H	39.398	0.852
MMLV-II Q299I	39.183	1.155
MMLV-II Q299K	40.000	0.000
MMLV-II Q299L	39.474	0.743
MMLV-II Q299M	40.000	0.000
MMLV-II Q299N	40.000	0.000
MMLV-II Q299P	40.000	0.000
MMLV-II Q299R	40.000	0.000
MMLV-II Q299S	40.000	0.000
MMLV-II Q299T	40.000	0.000
MMLV-II Q299V	40.000	0.000
MMLV-II Q299W	40.000	0.000
MMLV-II Q299Y	40.000	0.000
MMLV-II T332A	39.087	1.291
MMLV-II T332C	38.956	1.476
MMLV-II T332D	40.000	0.000
MMLV-II T332E	39.554	0.631
MMLV-II T332F	40.000	0.000

MMLV-II T332G	37.321	2.009
MMLV-II T332H	39.215	1.110
MMLV-II T332I	39.344	0.927
MMLV-II T332K	40.000	0.000
MMLV-II T332L	40.000	0.000
MMLV-II T332M	37.775	1.632
MMLV-II T332N	37.326	0.834
MMLV-II T332P	40.000	0.000
MMLV-II T332Q	39.509	0.694
MMLV-II T332R	39.588	0.582
MMLV-II T332S	39.765	0.332
MMLV-II T332V	36.977	0.384
MMLV-II T332W	40.000	0.000
MMLV-II T332Y	40.000	0.000
MMLV-II V433A	40.000	0.000
MMLV-II V433C	37.504	0.682
MMLV-II V433D	40.000	0.000
MMLV-II V433E	35.189	0.336
MMLV-II V433F	39.379	0.878
MMLV-II V433G	39.482	0.732
MMLV-II V433H	40.000	0.000
MMLV-II V433I	39.781	0.310
MMLV-II V433K	35.770	0.623
MMLV-II V433L	39.015	0.744
MMLV-II V433M	39.119	1.247
MMLV-II V433N	33.981	0.185
MMLV-II V433P	40.000	0.000
MMLV-II V433Q	40.000	0.000
MMLV-II V433R	37.230	1.247
MMLV-II V433S	37.850	0.846
MMLV-II V433T	37.564	1.895
MMLV-II V433W	37.770	1.622
MMLV-II V433Y	40.000	0.000
MMLV-IV	26.102	0.033

Table 28. Two-Step cDNA Synthesis by MMLV-RT single mutants using random priming. The data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Ct Mean	Ct Standard Deviation
MMLV-II	40.000	0.000
MMLV-II I593A	40.000	0.000
MMLV-II I593C	40.000	0.000
MMLV-II I593D	39.992	0.012
MMLV-II I593E	40.000	0.000
MMLV-II I593F	39.189	1.147

MMLV-II I593G	40.000	0.000
MMLV-II I593H	40.000	0.000
MMLV-II I593K	40.000	0.000
MMLV-II I593L	40.000	0.000
MMLV-II I593M	40.000	0.000
MMLV-II I593N	40.000	0.000
MMLV-II I593P	40.000	0.000
MMLV-II I593Q	39.201	0.853
MMLV-II I593R	38.928	1.516
MMLV-II I593S	39.025	1.379
MMLV-II I593T	38.385	1.227
MMLV-II I593V	39.574	0.603
MMLV-II I593W	32.572	0.054
MMLV-II I593Y	40.000	0.000
MMLV-II L280A	40.000	0.000
MMLV-II L280C	40.000	0.000
MMLV-II L280D	40.000	0.000
MMLV-II L280E	40.000	0.000
MMLV-II L280F	40.000	0.000
MMLV-II L280G	40.000	0.000
MMLV-II L280H	40.000	0.000
MMLV-II L280I	34.152	0.276
MMLV-II L280K	40.000	0.000
MMLV-II L280M	39.973	0.038
MMLV-II L280N	40.000	0.000
MMLV-II L280P	40.000	0.000
MMLV-II L280Q	40.000	0.000
MMLV-II L280R	40.000	0.000
MMLV-II L280S	40.000	0.000
MMLV-II L280T	40.000	0.000
MMLV-II L280V	39.260	1.046
MMLV-II L280W	40.000	0.000
MMLV-II L280Y	40.000	0.000
MMLV-II L82A	40.000	0.000
MMLV-II L82C	40.000	0.000
MMLV-II L82D	40.000	0.000
MMLV-II L82E	39.672	0.463
MMLV-II L82F	36.854	0.708
MMLV-II L82G	40.000	0.000
MMLV-II L82H	37.705	0.557
MMLV-II L82I	39.231	1.087
MMLV-II L82K	39.437	0.443
MMLV-II L82M	40.000	0.000
MMLV-II L82N	40.000	0.000
MMLV-II L82P	40.000	0.000

MMLV-II L82Q	40.000	0.000
MMLV-II L82R	38.595	1.191
MMLV-II L82S	40.000	0.000
MMLV-II L82T	38.449	1.192
MMLV-II L82V	39.438	0.795
MMLV-II L82W	39.178	1.163
MMLV-II L82Y	36.758	0.962
MMLV-II Q299A	40.000	0.000
MMLV-II Q299C	40.000	0.000
MMLV-II Q299D	38.003	1.414
MMLV-II Q299E	39.338	0.936
MMLV-II Q299F	40.000	0.000
MMLV-II Q299G	40.000	0.000
MMLV-II Q299H	40.000	0.000
MMLV-II Q299I	39.850	0.212
MMLV-II Q299K	40.000	0.000
MMLV-II Q299L	40.000	0.000
MMLV-II Q299M	40.000	0.000
MMLV-II Q299N	40.000	0.000
MMLV-II Q299P	40.000	0.000
MMLV-II Q299R	40.000	0.000
MMLV-II Q299S	40.000	0.000
MMLV-II Q299T	40.000	0.000
MMLV-II Q299V	40.000	0.000
MMLV-II Q299W	40.000	0.000
MMLV-II Q299Y	40.000	0.000
MMLV-II T332A	39.814	0.264
MMLV-II T332C	40.000	0.000
MMLV-II T332D	40.000	0.000
MMLV-II T332E	40.000	0.000
MMLV-II T332F	40.000	0.000
MMLV-II T332G	38.897	1.560
MMLV-II T332H	40.000	0.000
MMLV-II T332I	40.000	0.000
MMLV-II T332K	40.000	0.000
MMLV-II T332L	38.169	2.589
MMLV-II T332M	37.410	1.906
MMLV-II T332N	38.983	1.362
MMLV-II T332P	39.046	1.350
MMLV-II T332Q	40.000	0.000
MMLV-II T332R	40.000	0.000
MMLV-II T332S	40.000	0.000
MMLV-II T332V	38.650	1.326
MMLV-II T332W	40.000	0.000
MMLV-II T332Y	40.000	0.000

MMLV-II V433A	40.000	0.000
MMLV-II V433C	37.605	0.184
MMLV-II V433D	40.000	0.000
MMLV-II V433E	34.693	0.193
MMLV-II V433F	40.000	0.000
MMLV-II V433G	40.000	0.000
MMLV-II V433H	40.000	0.000
MMLV-II V433I	39.792	0.294
MMLV-II V433K	35.725	0.464
MMLV-II V433L	40.000	0.000
MMLV-II V433M	40.000	0.000
MMLV-II V433N	34.604	0.554
MMLV-II V433P	40.000	0.000
MMLV-II V433Q	38.844	1.001
MMLV-II V433R	38.817	0.839
MMLV-II V433S	38.202	1.372
MMLV-II V433T	37.573	0.623
MMLV-II V433W	37.611	1.690
MMLV-II V433Y	40.000	0.000
MMLV-IV	26.053	0.098

Table 29. One-Step cDNA Synthesis by MMLV-RT single mutants by gene specific priming. The data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Ct Mean	Ct Standard Deviation
MMLV-II	32.775	0.189
MMLV-II I593A	32.438	0.209
MMLV-II I593C	32.680	0.053
MMLV-II I593D	31.775	0.237
MMLV-II I593E	30.635	0.048
MMLV-II I593F	30.411	0.008
MMLV-II I593G	30.904	0.098
MMLV-II I593H	29.686	0.131
MMLV-II I593K	31.832	0.259
MMLV-II I593L	32.289	0.273
MMLV-II I593M	32.162	0.078
MMLV-II I593N	31.410	0.251
MMLV-II I593P	34.728	0.201
MMLV-II I593Q	31.609	0.032
MMLV-II I593R	31.144	0.133
MMLV-II I593S	30.548	0.247
MMLV-II I593T	29.572	0.236
MMLV-II I593V	30.673	0.142
MMLV-II I593W	28.179	0.092
MMLV-II I593Y	30.858	0.067

MMLV-II L280A	36.160	0.729
MMLV-II L280C	32.097	0.261
MMLV-II L280D	40.000	0.000
MMLV-II L280E	39.115	1.251
MMLV-II L280F	34.573	0.371
MMLV-II L280G	40.000	0.000
MMLV-II L280H	37.255	0.322
MMLV-II L280I	29.267	1.032
MMLV-II L280K	34.274	0.095
MMLV-II L280M	32.746	0.223
MMLV-II L280N	39.677	0.457
MMLV-II L280P	33.045	0.095
MMLV-II L280Q	39.190	1.145
MMLV-II L280R	40.000	0.000
MMLV-II L280S	40.000	0.000
MMLV-II L280T	37.074	0.325
MMLV-II L280V	30.461	0.052
MMLV-II L280W	40.000	0.000
MMLV-II L280Y	40.000	0.000
MMLV-II L82A	31.729	0.308
MMLV-II L82C	31.131	0.192
MMLV-II L82D	34.280	0.227
MMLV-II L82E	32.973	0.430
MMLV-II L82F	29.760	0.030
MMLV-II L82G	33.066	0.217
MMLV-II L82H	30.098	0.078
MMLV-II L82I	31.605	0.083
MMLV-II L82K	29.258	0.015
MMLV-II L82M	30.280	0.027
MMLV-II L82N	33.074	0.323
MMLV-II L82P	38.754	1.762
MMLV-II L82Q	32.001	0.164
MMLV-II L82R	30.208	0.128
MMLV-II L82S	31.841	0.231
MMLV-II L82T	28.908	0.044
MMLV-II L82V	29.533	0.057
MMLV-II L82W	29.580	0.056
MMLV-II L82Y	28.934	0.073
MMLV-II Q299A	31.113	0.138
MMLV-II Q299C	35.953	0.542
MMLV-II Q299D	32.292	0.080
MMLV-II Q299E	31.663	0.027
MMLV-II Q299F	36.143	0.317
MMLV-II Q299G	31.929	0.131
MMLV-II Q299H	32.387	0.133

MMLV-II Q299I	37.763	1.582
MMLV-II Q299K	32.326	0.096
MMLV-II Q299L	34.807	0.180
MMLV-II Q299M	32.514	0.375
MMLV-II Q299N	34.040	0.186
MMLV-II Q299P	39.460	0.764
MMLV-II Q299R	33.044	0.354
MMLV-II Q299S	33.438	0.256
MMLV-II Q299T	35.093	0.926
MMLV-II Q299V	35.114	1.045
MMLV-II Q299W	38.998	1.417
MMLV-II Q299Y	39.055	1.336
MMLV-II T332A	30.528	0.084
MMLV-II T332C	30.785	0.135
MMLV-II T332D	33.310	0.348
MMLV-II T332E	32.711	0.106
MMLV-II T332F	33.201	0.179
MMLV-II T332G	30.424	0.054
MMLV-II T332H	31.913	0.306
MMLV-II T332I	32.072	0.115
MMLV-II T332K	31.591	0.082
MMLV-II T332L	34.011	0.133
MMLV-II T332M	29.039	0.164
MMLV-II T332N	29.500	0.135
MMLV-II T332P	33.976	0.272
MMLV-II T332Q	31.599	0.041
MMLV-II T332R	32.950	0.130
MMLV-II T332S	31.003	0.341
MMLV-II T332V	29.835	0.061
MMLV-II T332W	35.431	0.099
MMLV-II T332Y	33.384	0.164
MMLV-II V433A	30.757	0.105
MMLV-II V433C	29.901	0.305
MMLV-II V433D	34.152	0.170
MMLV-II V433E	28.868	0.011
MMLV-II V433F	31.529	0.009
MMLV-II V433G	33.663	0.412
MMLV-II V433H	31.811	0.069
MMLV-II V433I	30.460	0.071
MMLV-II V433K	30.040	0.109
MMLV-II V433L	31.758	0.063
MMLV-II V433M	30.791	0.095
MMLV-II V433N	28.566	0.074
MMLV-II V433P	37.436	1.824
MMLV-II V433Q	30.586	0.104

MMLV-II V433R	30.773	0.080
MMLV-II V433S	29.768	0.074
MMLV-II V433T	29.096	0.107
MMLV-II V433W	29.130	0.064
MMLV-II V433Y	32.676	0.279
MMLV-IV	25.979	0.043

Table 30. Two-Step cDNA Synthesis by MMLV-RT stacked mutants using oligo dT priming. The data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Temperature (°C)	Ct Mean	Ct Standard Deviation
MMLV-II	42	25.207	0.025
MMLV-II	55	28.180	0.022
MMLV-II Q68R/Q79R/L99R/E282D	42	25.287	0.068
	55	26.442	0.044
MMLV-II Q68R/Q79R/L99R/E282D/V433R	42	25.344	0.065
	55	26.586	0.077
MMLV-II Q68R/Q79R/L99R/E282D/I593E	42	25.266	0.112
	55	27.389	0.069
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	42	25.357	0.087
	55	26.953	0.034
MMLV-II Q68R/Q79R/L82R/L99R/E282D	42	25.394	0.011
	55	27.171	0.028
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/I593E	42	25.371	0.061
	55	26.689	0.068
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/I593E	42	25.258	0.035
	55	26.979	0.034
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	42	25.171	0.006
	55	26.299	0.025
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	42	25.146	0.052
	55	26.320	0.036
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	42	25.176	0.044
	55	26.750	0.040
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	42	25.110	0.046
	55	26.587	0.049
MMLV-IV	42	25.184	0.025
MMLV-IV	55	25.153	0.037
SuperScript-IV	42	25.082	0.073
SuperScript-IV	55	25.080	0.047

Table 31. Two-Step cDNA Synthesis by MMLV-RT stacked mutants using random priming. The data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Temperature (°C)	Ct Mean	Ct Standard Deviation
MMLV-II	42	25.264	0.019
MMLV-II	55	28.443	0.014
MMLV-II Q68R/Q79R/L99R/E282D	42	25.399	0.040
	55	26.484	0.072
MMLV-II Q68R/Q79R/L99R/E282D/V433R	42	25.324	0.063
	55	26.794	0.065
MMLV-II Q68R/Q79R/L99R/E282D/I593E	42	25.278	0.025
	55	27.616	0.058
MMLV-II Q68R/Q79R/L99R/E282D/Q299E	42	25.281	0.079
	55	27.148	0.025
MMLV-II Q68R/Q79R/L82R/L99R/E282D	42	25.279	0.053
	55	27.243	0.008
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/I593E	42	25.409	0.065
	55	26.704	0.066
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/I593E	42	25.581	0.062
	55	26.605	0.028
MMLV-II Q68R/Q79R/L99R/E282D/Q299E/V433R/I593E	42	25.355	0.158
	55	26.305	0.066
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/V433R/I593E	42	25.418	0.120
	55	26.403	0.055
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/I593E	42	25.374	0.115
	55	26.747	0.065
MMLV-II Q68R/Q79R/L82R/L99R/E282D/Q299E/T332E/V433R/I593E	42	25.426	0.082
	55	26.481	0.017
MMLV-IV	42	25.394	0.162
MMLV-IV	55	25.185	0.022
SuperScript-IV	42	25.299	0.132
SuperScript-IV	55	25.214	0.021

Table 32. One-Step cDNA Synthesis by MMLV-RT stacked mutants by gene specific priming. The data was generated *via* qPCR human normalizer assay and data is reported by Ct value.

MMLV-RT Variant	Temperature (°C)	Concentration of RT (nM)	Ct Mean	Ct Standard Deviation
MMLV-II	50	0.28	26.401	0.022
		1.4	24.701	0.061
		7.0	24.664	0.007
	60	0.28	31.134	0.205

		1.4	28.109	0.042
		7.0	27.644	0.061
MMLV-II Q68R/Q79R/L99R/E28 2D	50	0.28	25.171	0.046
		1.4	24.440	0.037
		7.0	24.406	0.010
	60	0.28	28.848	0.114
		1.4	25.905	0.066
		7.0	25.618	0.057
MMLV-II Q68R/Q79R/L99R/E28 2D/V433R	50	0.28	24.967	0.068
		1.4	24.386	0.015
		7.0	24.433	0.079
	60	0.28	28.516	0.051
		1.4	25.803	0.063
		7.0	25.620	0.035
MMLV-II Q68R/Q79R/L99R/E28 2D/I593E	50	0.28	24.660	0.053
		1.4	24.377	0.028
		7.0	24.355	0.021
	60	0.28	27.488	0.074
		1.4	25.413	0.049
		7.0	25.209	0.136
MMLV-II Q68R/Q79R/L99R/E28 2D/Q299E	50	0.28	25.044	0.094
		1.4	24.422	0.023
		7.0	24.528	0.055
	60	0.28	28.818	0.137
		1.4	25.953	0.082
		7.0	25.754	0.098
MMLV-II Q68R/Q79R/L82R/L99 R/E282D	50	0.28	25.014	0.152
		1.4	24.467	0.020
		7.0	24.507	0.046
	60	0.28	28.743	0.076
		1.4	26.662	0.012
		7.0	25.883	0.022
MMLV-II Q68R/Q79R/L99R/E28 2D/Q299E/I593E	50	0.28	24.771	0.027
		1.4	24.501	0.008
		7.0	24.485	0.087
	60	0.28	27.721	0.057
		1.4	25.836	0.030
		7.0	25.199	0.016
MMLV-II Q68R/Q79R/L82R/L99 R/E282D/Q299E/I593 E	50	0.28	24.777	0.029
		1.4	24.432	0.033
		7.0	24.435	0.024
	60	0.28	27.854	0.035
		1.4	25.613	0.028
		7.0	25.072	0.030
MMLV-II Q68R/Q79R/L99R/E28 2D/Q299E/V433R/I59 3E	50	0.28	24.550	0.003
		1.4	24.333	0.033
		7.0	24.345	0.030
	60	0.28	26.399	0.051

		1.4	25.236	0.040
		7.0	25.105	0.050
MMLV-II Q68R/Q79R/L82R/L99 R/E282D/Q299E/V433 R/I593E	50	0.28	24.562	0.047
		1.4	24.350	0.039
		7.0	24.302	0.015
	60	0.28	26.459	0.022
		1.4	25.247	0.069
		7.0	25.001	0.050
MMLV-II Q68R/Q79R/L82R/L99 R/E282D/Q299E/T332 E/I593E	50	0.28	24.614	0.047
		1.4	24.420	0.051
		7.0	24.361	0.021
	60	0.28	26.769	0.089
		1.4	25.609	0.041
		7.0	25.348	0.043
MMLV-II Q68R/Q79R/L82R/L99 R/E282D/Q299E/T332 E/V433R/I593E	50	0.28	24.594	0.075
		1.4	24.402	0.045
		7.0	24.291	0.057
	60	0.28	26.591	0.018
		1.4	25.517	0.048
		7.0	25.193	0.027
MMLV-IV	50	0.28	24.397	0.091
		1.4	24.303	0.062
		7.0	24.189	0.039
	60	0.28	25.807	0.045
		1.4	25.180	0.037
		7.0	24.625	0.011
SuperScript-IV	50	0.28	24.743	0.049
		1.4	24.213	0.017
		7.0	24.008	0.036
	60	0.28	26.124	0.103
		1.4	24.681	0.070
		7.0	24.180	0.082

Table 33. Sequences of quadruple or more mutant MMLV RTase variants.

SEQ ID NO:	Construct	Construct Sequence (AA)
686	MMLV-II Q68R/Q79R/L99 R/E282D/V433R	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS I KQYPMSREA RLGIKPHIRRLLDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKD A FFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQH PDL I LLQYVDDLLLAATSELD CQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLG YLLKEGQRWL TDARKETVMGQPTPKTPRQLREFLGTAGFCRLWIP GFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQA

		LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLRILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSLLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNSTRYAFATAHIHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
687	MMLV-II Q68R/Q79R/L99 R/E282D/I593E	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS IKQYPM SREA RLGIKPHIRRLDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREV NKRVEDIHPTVNPYNLLSGLP PSHQWYTVLDLKD AFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLG YLLKEGQRWL TDARKETVMGQPTPKTPRQLREFLG TAGFCRLWIP GFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSLLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNSTRYAFATAHEHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
688	MMLV-II Q68R/Q79R/L99 R/E282D/Q299E	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS IKQYPM SREA RLGIKPHIRRLDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREV NKRVEDIHPTVNPYNLLSGLP PSHQWYTVLDLKD AFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLG YLLKEGQRWL TDARKETVMGQPTPKTPRELREFLG TAGFCRLWIP GFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSLLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNSTRYAFATAHIHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF

689	MMLV-II Q68R/Q79R/L99 R/E282D/T332E	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS IKQYPMSREA RLGIKPHIRRLDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGILLKEGQRWL TDARKETVMGQPTPKTPRQLREFLG TAGFCRLWIP GFAEMAAPLYPLTKTGELFNWGPDQQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSSLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNSTRYAFATAHIHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
690	MMLV-II Q68R/Q79R/L99 R/L280R	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS IKQYPMSREA RLGIKPHIRRLDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGILLKEGQRWR TEARKETVMGQPTPKTPRQLREFLG TAGFCRLWIP GFAEMAAPLYPLTKGTGFN WGPDQQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSSLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNSTRYAFATAHIHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
691	MMLV-II Q68R/Q79R/L99 R/L280R/E282D	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS IKQYPMSREA RLGIKPHIRRLDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGILLKEGQRWR TDARKETVMGQPTPKTPRQLREFLG TAGFCRLWIP GFAEMAAPLYPLTKGTGFN WGPDQQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL

		GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSLLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHIHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
692	MMLV-II Q68R/L82R/L99 R/E282D	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVSIKQYPMSREA RLGIKPHIQRLRDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREVNRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKD AFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLG YLLKEGQRWL TDARKETVMGQPTPKT PRQLREFLG TAGFCRLWIP GFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSLLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHIHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
693	MMLV-II Q68R/Q79R/L82 R/L99R/E282D	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVSIKQYPMSREA RLGIKPHIRRLRDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREVNRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKD AFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLG YLLKEGQRWL TDARKETVMGQPTPKT PRQLREFLG TAGFCRLWIP GFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSLLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHIHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
694	MMLV-II Q68R/Q79R/L99	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVSIKQYPMSREA

	R/E282D/Q299E /I593E	RLGIKPHIRRLDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREVNRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGILLKEGQRWL TDARKETVMGQPTPKTPRELREFLGTAFCRLWIP GFAEMAAPLYPLTKTGTLFNWGPDDQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTG SSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHEHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
695	MMLV-II Q68R/Q79R/L82 R/L99R/E282D/ Q299E/I593E	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS IKQYPM SREA RLGIKPHIRRLDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREVNRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGILLKEGQRWL TDARKETVMGQPTPKTPRELREFLGTAFCRLWIP GFAEMAAPLYPLTKTGTLFNWGPDDQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTG SSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNTRYAFATAHEHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
696	MMLV-II Q68R/Q79R/L99 R/E282D/Q299E /V433R/I593E	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS IKQYPM SREA RLGIKPHIRRLDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREVNRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGILLKEGQRWL TDARKETVMGQPTPKTPRELREFLGTAFCRLWIP GFAEMAAPLYPLTKTGTLFNWGPDDQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLRILAPHAVEALVKQPPDRWLSNA

		RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSLLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNSTRYAFATAHEHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
697	MMLV-II Q68R/Q79R/L82 R/L99R/E282D/ Q299E/V433R/I 593E	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS IKQYPM SREA RLGIKPHIRRLRDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREV NKRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKD AFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLYLLKEGQRWL TDARKETVMGQPTPKTPRELREFLG TAGFCRLWIP GFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLRILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSLLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNSTRYAFATAHEHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
698	MMLV-II Q68R/Q79R/L82 R/L99R/E282D/ Q299E/T332E/I5 93E	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS IKQYPM SREA RLGIKPHIRRLRDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREV NKRVEDIHPTVPNPYNLLSGLP PSHQWYTVLDLKD AFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLYLLKEGQRWL TDARKETVMGQPTPKTPRELREFLG TAGFCRLWIP GFAEMAAPLYPLTKTGELFNWGPDQQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTGG SSLLQEGQRKAGAAVTTETETEWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNSTRYAFATAHEHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
699	MMLV-II Q68R/Q79R/L82 R/L99R/E282D/ Q299E/T332E/V	TLNIEDEHRLHETSKEPDVSLGSTWLSDFPQAWAE TGGMGLAVRQAPLI I PLKATSTPVS IKQYPM SREA RLGIKPHIRRLRDQGILVPCQSPWNTPLRPVKKPG TNDYRPVQDLREV NKRVEDIHPTVPNPYNLLSGLP

	433R/I593E	PSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDP EMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADF RIQHPDLILLQYVDDLLLAATSELDCCQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWL TDARKETVMGQPTPKTPRELREFLGTAGFCRLWIP GFAEMAAPLYPLTKTGELFNWGPDQQKAYQEIKQA LLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLT KDAGKLTMGQPLRILAPHAVEALVKQPPDRWLSNA RMTHYQALLLDTRVQFGPVVALNPATLLPLPEEG LQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTG SSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQR AQLIALTQALKMAEGKKLVYTNSTRYAFATAHEHG EIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRL SIIHCPGHQKGHSAEARGNRMADQAARKAAITETP DTSTLLIENSSPYTSEHF
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WHAT IS CLAIMED IS:

Claim 1: An isolated Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutant comprising the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least one amino acid substitution that is:

- (a) an isoleucine to arginine substitution at position 61 (I61R);
- (b) a glutamine to arginine substitution at position 68 (Q68R);
- (c) a glutamine to arginine substitution at position 79 (Q79R);
- (d) a leucine to arginine substitution at position 99 (L99R);
- (e) a glutamic acid to aspartic acid substitution at position 282 (E282D);
- (f) an arginine to alanine substitution at position 298 (R298A);
- (g) a glutamine to glutamic acid substitution at position 299 (Q299E);
- (h) a threonine to glutamic acid substitution at position 332 (T332E);
- (i) a valine to arginine substitution at position 433 (V433R); and/or
- (j). a isoleucine to glutamic acid substitution at position 593 (I593E).

Claim 2: The isolated MMLV RTase mutant of claim 1, wherein the MMLV RTase mutant comprises an amino acid sequence as set forth in any one of SEQ ID NOs: 637-699.

Claim 3: The isolated MMLV RTase mutant of claim 2, wherein the MMLV RTase mutant comprises an amino acid sequence as set forth in SEQ ID NO: 674.

Claim 4: An isolated Moloney murine leukemia virus (MMLV) reverse transcriptase (RTase) mutant comprising the amino acid sequence of SEQ ID NO: 637, wherein the amino acid sequence of the MMLV RTase mutant further comprises at least two amino acid substitutions that are:

- (a) an isoleucine to arginine substitution at position 61 and a glutamic acid to aspartic acid substitution at position 282 (I61R/E282D);
- (b) a leucine to arginine at substitution position 99 and a glutamic acid to aspartic acid substitution at position 282 (L99R/E282D);
- (c) a glutamine to arginine substitution at position 68 and a glutamic acid to aspartic acid substitution at position 282 (Q68R/E282D);

- (d) a glutamine to arginine substitution at position 79 and a glutamic acid to aspartic acid substitution at position 282 (Q79R/E282D);
- (e) a glutamic acid to aspartic acid substitution at position 282 and an arginine to alanine substitution at position 298 (E282D/R298A);
- (f) an isoleucine to arginine substitution at position 61 and a leucine to arginine substitution at position 99 (I61R/L99R);
- (g) an isoleucine to arginine substitution at position 61 and a glutamine to arginine substitution at position 68 (I61R/Q68R);
- (h) an isoleucine to arginine substitution at position 61 and a glutamine to arginine substitution at position 79 (I61R/Q79R);
- (i) an isoleucine to arginine substitution at position 61 and an arginine to alanine substitution at position 298 (I61R/R298A);
- (j) a glutamine to arginine substitution at position 68 and a leucine to arginine substitution at position 99 (Q68R/L99R);
- (k) a glutamine to arginine substitution at position 79 and a leucine to arginine substitution at position 99 (Q79R/L99R);
- (l) a leucine to arginine at substitution position 99 and an arginine to alanine substitution at position 298 (L99R/R298A);
- (m) a glutamine to arginine substitution at position 68 and a glutamine to arginine substitution at position 79 (Q68R/Q79R);
- (n) a glutamine to arginine substitution at position 68 and an arginine to alanine substitution at position 298 (Q68R/R298A); or
- (o) a glutamine to arginine substitution at position 79 and an arginine to alanine substitution at position 298 (Q79R/R298A).

Claim 5: The isolated MMLV RTase mutant of claim 4, wherein the MMLV RTase mutant comprises the amino acid sequence of one or more of SEQ ID NOs: 637-699.

Claim 6: The MMLV RTase mutant of either claim 1 or 4, wherein the MMLV RTase mutant lacks RNase H activity.

Claim 7: The MMLV RTase mutant of either claim 1 or 4, wherein the MMLV RTase mutant possesses at least one of the following characteristics: enhanced DNA synthesis, increased fidelity, or enhanced thermostability.

Claim 8: An isolated nucleic acid molecule comprising a nucleotide sequence encoding the MMLV RTase mutant of either claim 1 or 4.

Claim 9: A composition comprising the isolated MMLV RTase mutant of either claim 1 or 4.

Claim 10: The composition of claim 9, wherein the isolated MMLV RTase mutant lacks RNase H activity.

Claim 11: The composition of claim 9, wherein the isolated MMLV RTase mutant possesses at least one of the following characteristics: enhanced DNA synthesis, increased fidelity, or enhanced thermostability.

Claim 12: A kit comprising the isolated MMLV RTase mutant of either claim 1 or 4.

Claim 13: The kit of claim 12, wherein the isolated MMLV RTase mutant lacks RNase H activity.

Claim 14: The kit of claim 12, wherein the isolated MMLV RTase mutant possesses at least one of the following characteristics: enhanced DNA synthesis, increased fidelity, or enhanced thermostability.

Claim 15: A method for synthesizing complementary deoxyribonucleic acid (cDNA) comprising:

- (a) providing the isolated MMLV RTase mutant of either claim 1 or 4; and
- (b) contacting the isolated MMLV RTase mutant with a nucleic acid template to permit synthesis of cDNA.

Claim 16: A method for performing reverse transcription-polymerase chain reaction (RT-PCR) comprising:

- (a) providing the isolated MMLV RTase mutant of either claim 1 or 4; and
- (b) contacting the isolated MMLV RTase mutant with a nucleic acid template to replicate and amplify the nucleic acid template.