

Supplemental Information

A truncated reverse transcriptase enhances prime editing by split AAV vectors

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SUPPLEMENTARY INFORMATION

Supplemental Figures

Figure S1

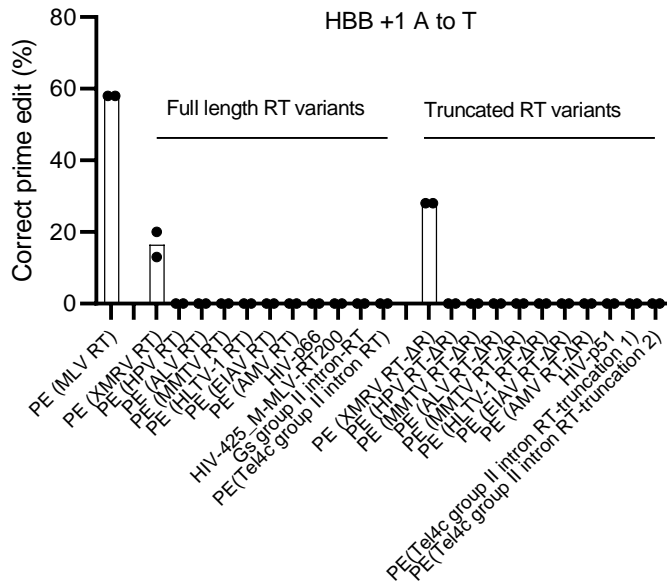


Figure S1. Screening of RT variants for prime editing activity. The RT variants were human codon-optimized by GenScript and used to replace the M-MLV RT to form PE variants. PE constructs were transfected together with plasmids encoding the pegRNA and ngRNA into HEK293T cells and prime editing results were analyzed after 3 days. The data are presented as mean values with all data points from independent experiments shown.

Figure S2

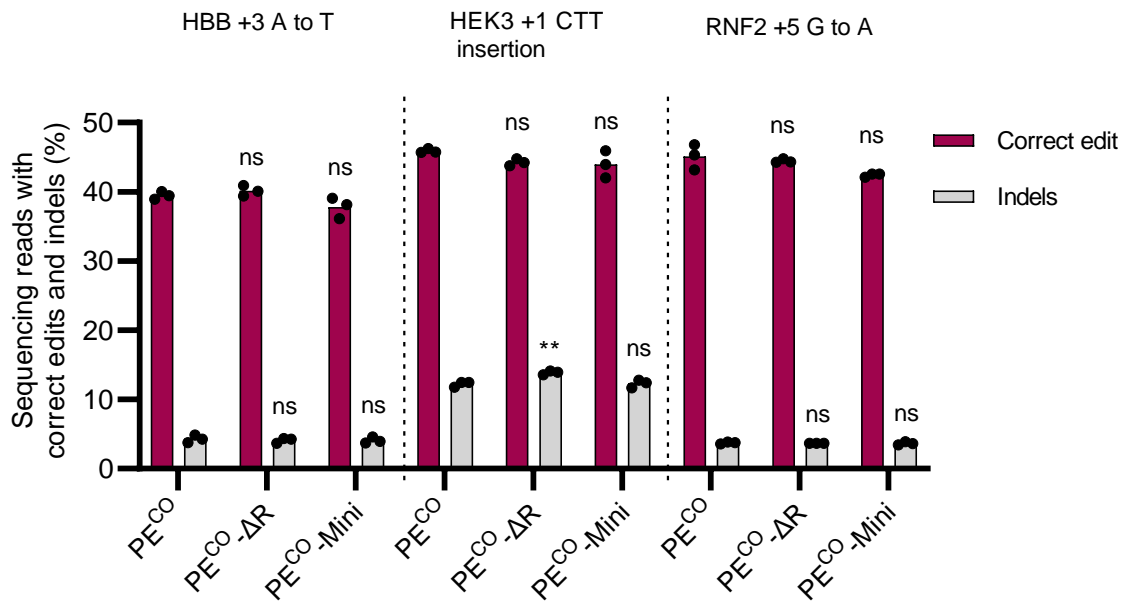


Figure S2. PE^{CO}, PE^{CO}-ΔR, and PE^{CO}-Mini have the same prime editing outcomes. NGS was performed to quantify the frequency of correct prime edits and indels. The bars show mean values with all data points shown for biological replicates. ns, not significant; **, p < 0.01.

Figure S3

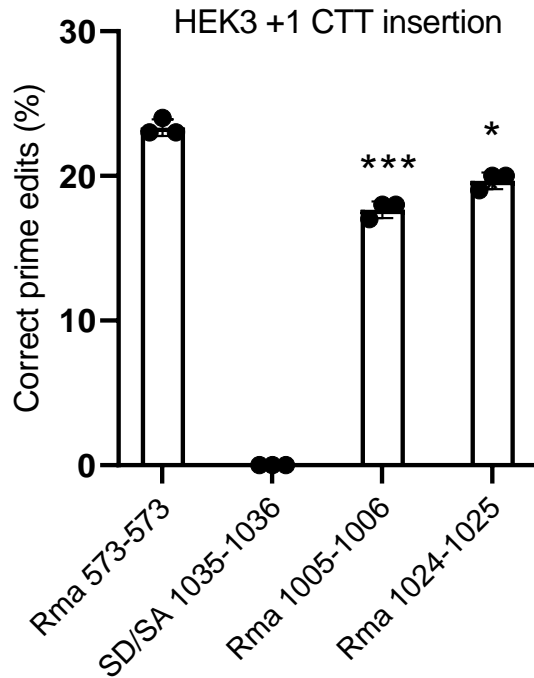


Figure S3. Comparison of Rma 573-574 with other reported split sites. The split constructs were made using the same plasmid backbone, and a same molar amount of these plasmids were transfected into HEK293T cells together with equal amounts of pegRNA and ngRNA plasmids. Prime editing efficiencies were determined by ICE analysis of Sanger sequencing chromatograms. The bars represent means with data points from the biological replicates shown. *, $p < 0.05$; ***, $p < 0.001$.

Figure S4

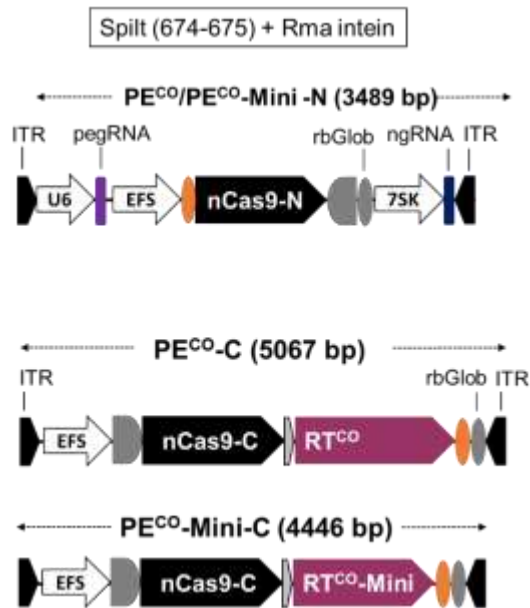


Figure S4. Schematic of the AAV vectors carrying the split PE system. The sizes of the AAV vector genomes including the ITRs are illustrated schematically. To prevent recombination, we used two different RNA Pol III promoters (U6 and 7SK) to drive pegRNA and ngRNA expression, respectively. Also, an optimized gRNA scaffold with some sequence modifications were used for the pegRNA.¹ EFS: EF-1 Alpha Short promoter.

Figure S5

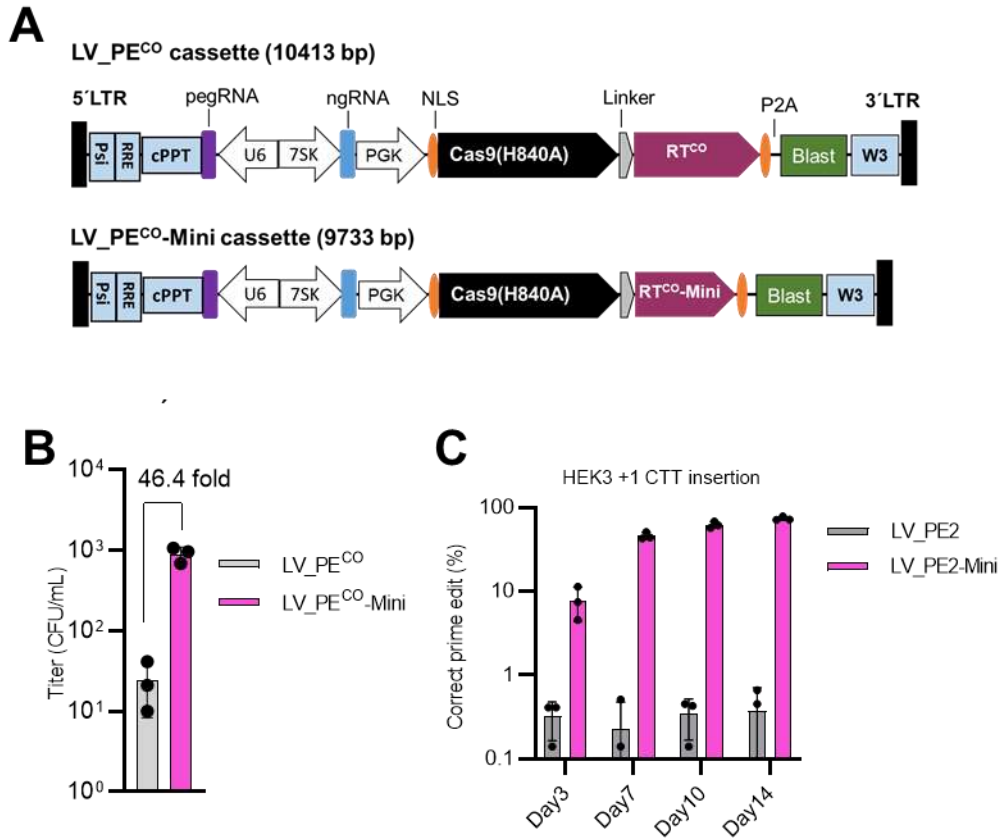


Figure S5. PE^{CO}-Mini provides an LV titer advantage over PE^{CO}. (A) Schematic of LV vectors encoding all-in-one PE^{CO}-Mini or PE^{CO} systems. Psi, packaging signal; RRE, Rev response elements; cPPT, central polypurine tract. (B) Titer measurement of LV_{PE^{CO}} and LV_{PE^{CO}-Mini}. (C) Prime editing efficiencies of LV_{PE^{CO}} and LV_{PE^{CO}-Mini} in HEK293T cells. See Materials and Methods section for experimental details. Bars represent means \pm SD with individual data points shown for the biological replicates.

Figure S6

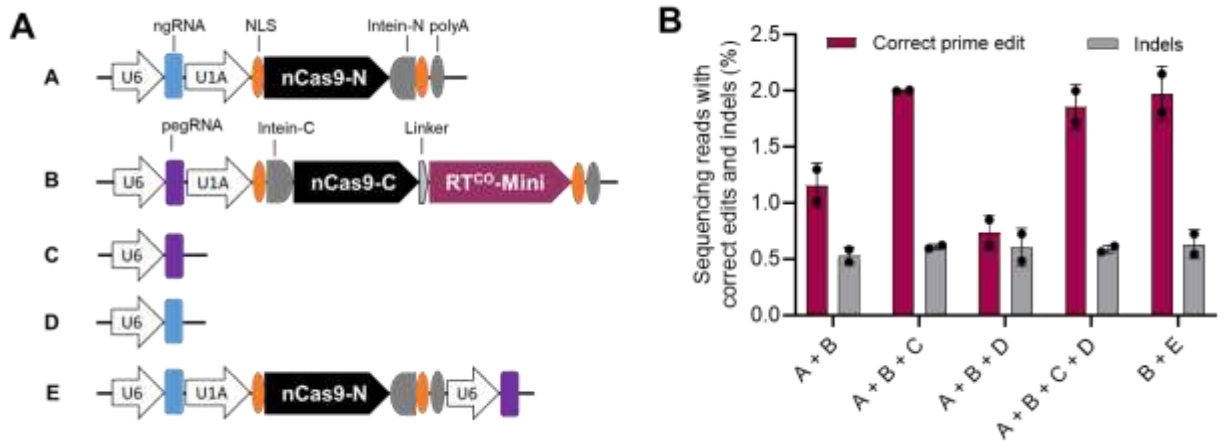


Figure S6. pegRNA expression cassettes in both split AAV vectors enhances prime editing.

(A) Schematic figure of plasmids harboring PE expression cassette(s). (B) Prime editing analysis of different plasmid combinations for +1A insertion in Hepa 1-6 cells. Cells were transfected with plasmids and after 3 days the cells were subjected to prime editing analysis by NGS. Bars represent means \pm SD with individual data point shown for the biological replicates.

1. Dang, Y., Jia, G., Choi, J., Ma, H., Anaya, E., Ye, C., Shankar, P., and Wu, H. (2015). Optimizing sgRNA structure to improve CRISPR-Cas9 knockout efficiency. *Genome biology* 16, 1-10.

Supplemental Data 1: RT variants from different species

HIV-p51 (1320 bp)

CCTATCAGCCCCATCGAGACAGTGCCTGAAAGCTGAAACCTGGAATGGATGGCCCTAAAGTGAAGCAATGGCCTCTGACCGAGGAAAAGATCAAG
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HIV-p66 (1680 bp)

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GGAGCTGGTGAACAGATCATCGAACAGCTGATCAAGAAGGAAAAGGTCTACCTGGCTTGGTCCCCGCTCATAAGGGAATTGGCGGCAACGAGC
AGGTGGATAAGCTGGTGTCCCGGCATCCGGAAGTGTG

HIV-425 _MLV-RT-200 (1275bp)

CCTATCAGCCCCATCGAGACAGTGCCTGAAAGCTGAAACCTGGAATGGATGGCCCTAAAGTGAAGCAATGGCCTCTGACCGAGGAAAAGATCAAG
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HPV RT (1827 bp); HPV RT-ΔR (1362 bp)

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MMTV (Mouse mammary tumor virus) RT (1725 bp); MMTV RT-ΔR (1338 bp)

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ALV(Avian leukosis virus) RT; ALV RT-ΔR (1335 bp)

ACAGTGGCTCTGCACCTGGCCATTCTCTGAAGTGGAAAGCCCGATCACACCCTGTGTGGATCGATCAGTGGCCCTGCTGCCTGAGGGAAAGTGGTGGC
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XMRV(Xenotropic MuLV-related virus) RT; XMRV RT-ΔR (1542 bp)

ACCTGAACATCGAGGACGAGTACCAGGCTGCAGAGACAAGCAAGAACCCGATGTGCCTCTGGGCGAGCACTGGCTGTCTGATTTTCCACAGGCCCT
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 AGGCAACAGAATGGCCGATCAGGCCGCTAGAGAAGCCCATGAAGGCCGTGGTGAACCTTCTACTGCTGCT

HLTV-1(Human T-cell leukemia virus1) RT; HLTV-1 RT-ΔR (1317 bp)

GGCTGGAACATCTGCTAGACACCTGAGACTCAGCAGTTCCTCTGAACCCCGAGAGACTGCAGGCTCTGCAGCATCTGTTTCGGAAGCCCTGG
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TGCACCACGTGGGAGCCACCAACCTGCCTGATCATGCAAGCTGAAGCCCTGACAGACGCCCTGCTGATCACCCCTATTCTGCAGCT

EIAV(Equine infectious anemia virus) RT; EIAV RT-ΔR (1260 bp)

ATCGAGCTGAAAAGAGGGGACAATGGGCCCTAAGATCCCTCAGTGGCCCTGCACCAAGAGAAAGCTGGAAAGGGCCAAAGAAAACCGTGCAGAGACTG
CTGAGCGAGGGCAAGACTCAGCGAGGCCAGGACAACAACCCCTACAACAGCCCATCTTCGTGATCAAAAAGCGGAGCGGCAAGTGGCGGCTGCTG
CAGGATCTGAGAGAACTGAACAAGACCGTGAAGTGGGCACCGAGATCTCCAGAGGACTTCTCATCCTGGCGCCTGATCAAGTGAAGCACATGA
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ATGTGGGAGATGCAGAAAGGATGGTACTACAGCTGGCTGCCCGAGATCGTGTACACCCACAGAGTGGTGCACGACGAGTGGCGGATGAAGCTGGT
GAAGAACACTACGAGCGGATCACCATCTACACCGATGGCGGAAGCAGAACCGGAGGGAATTCGCGCTACGTTGACCTTCAACGGCAGGACCAAG
CAGAAAAGACTGGGCCCCGTCACACATCAGGTGGCCGAAAGAATGGCCATCCAGTGGCCCTGGAAGATACCCCGGACAAAGCAAGTGAACATCGTG
ACCGACAGCTACTGCTGGAAGAACATCACCGAAGGCTGGGCTCGAGGGACCTCAAAAATCTTGGTGGCCCATCATCCAGAAGATCCCGGAGAG
AAGAAATCGTACTTGCCTGGTGCCTGGCCACAAGGGCTACTATGGAAATCAGTGGCCGACGAGGCCCAAGATCAAAGAGGAAATATGTCT
GGCTACCGAGGGGACGAAATCAAAGAAAAGCGGACGAGGAGCTGCGCTTCAGCTTGTGTGCCCTACGATATCATGATCCCCGTGTCCGACACC
AAGATCATCCCCACCGAGCTGAAGATCCAGTTCCACCTAACAGCTTCGGCTGGGTACAGGCAAGAGCAGCATGGCCAAACAGGGACTGCTGATCA
ACGGCGGCATCATCGACAGGGCTACACCGCGAGATCCAAGTCACTGCACCAACATCGGCAAGTCCAACATCAAGCTGATCGAGGGCCAGAAGT
TCGCCAGCTCATATCTCCAGCACACGAACAGCAGACAGCCCTGGGACGAGAGAAAGATCTCCAGAGAGGGCACAAGGCTTCCGAGCA
CTGGCGTGTCTGGGTGAGAAATATCCAAGAGGCTCAGGACGAGCAGGAACTGGCACACAAGCCCAAGATCTGGCCAGAACTACAAGATCC
CACTGACCGTGGCCAAGCAGATCACCAAGAA

AMV(Avian myeloblastosis virus) RT; AMV RT-ΔR (1338 bp)

ACAGTGGCCCTGCACCTGGCCATCCCCCTGAAGTGGAAAGCCAAACCACACCCAGTGTGGATCGACCAGTGGCCCTCCCCGAGGGCAAACTGGTGG
CCCTGACCCAGCTGGTGGAAAAGGAGTGCACCTGGGCGACATCGAGCCTTCTCTAGCTGTGGAATACCCCTGTCTTCGTGATCAGAAAAGGCCAG
CGGCTCTTACAGACTGTGATCTGAGAGCAGTAAATGCCAAGCTGGTCCCTTCCGTGCCGTCAGCAGGGCGCTCTGTGCTCCGCCCTG
CCAGACTTGGCCTCTGATGGTGTGATCTGAAGGACTGCTTTCTTCTATCCCTTGGCCGAGCAGGACAGAGAGGCGCTTCCGCTTTACTGCT
TCTGTGAACAACCAGGCTCCAGCTCGCAGATTCAGTGGAAAGTGTCTCCCGAGGGGATGACTGTAGCCCAACATCTGCCAGCTGATCGTGGGCC
AGATCTTGAACCACTGCGGTGAAGCACCCAGCCTGAGAATGTGCACTACATGGACGACCTGTGCTTGGCGCTTACTAGCCACGACGGCCTCGA
GGCCCGCGGAGGAAGTATCTCCACACTGGAAGAGCGGATTCACCATAGCCCTGATAAAGGTGACGCGGGAACCTGGCGTTCAATACCTGGCC
TATAAGCTGGGAAGCAGTACGTGGCCCTGTCCGCTGTGGCAGAACCTAGAATCGCTACACTGTGGGACGTGCAGAAAGCTGTGGGCAGCCTGC
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GCCCGGTGCGAACAGGGAGCTATCGCCGTGCTGGCGTGGCCAGGACTGAGCACCACCCAGACCTTGCCTGTGGCTTTACGACACACAGCTACCAAGG
CCTTACCGCCTGGCTGGAAGTGTGACTGACTGCTGATCAACAGCTGCGGGCCAGCCTGCGGACCTTCGGCAAGGAAGTGCACATCTGCTACT
GCCTGCTGTTTAGAGATGATCTGCCACTGCTGAGGGATCTCTGCTGCTGAGAGGCTTTGCCGCAAGATCCGGAGCAGCAGCCCTAGCA
TCTTGCATATCGCCAGACCTTGCACGTGTCCCTGAAGGTGGCGGTGACCGACCCCTGGCCTGGACCTACCGTGTGTTACCGACGCCTCATTAGC
ACCCACAAGGGCGTGGTGGTGTGGAGGGAAGGCCCCGTTGGGAGATTAAAGAAATCGCCGACCTGGGCGTAGCGTGAACAACCTGGAGGCCAGA
GCCGTGGCCATGGCCCTCCTGCTGTGGCCTACCACCCCTACAACTGGTTACAGACAGCGCTTCTGTTGGCAAGATGTGTTGAAAATGGGCCAGG
AGGGAGTGCCTAGCACCAGCCTGCTTTTCATCTCGAGGACGCCCTGTCTCAGAGAAGCGCTATGGCCGCGTGTGATGTGGGTCCACAGCGA
GGTGGCCGCTTTTACCAGAGGCAACGACGTGGCCGATAGCCAGGCCACATTCAGGCCCTAC

Gs group II intron-RT (1260 bp)

ATGGCCCTGCTGGAGCGGATCCTGGCCAGAGACAACCTGATCACCGCTCTGAAGCGGGTGGAGGCAATCAGGGCGCCCAGGCATCGACGGCGTCT
CCACAGATCAGCTGCGGATACATCCGGGCCATTGGAGCACCATCCACGCTCAGCTGCTGCTGGCACATACCCCTCTGCCCGGTGCGGAGAGT
GAGATTCCTAAGCCCGCGGAGGAACAAGACAGCTGGGAATCCCCACCGTGGTTGATCGCTGATCCAGCAGGCCATCTGCAGGAGCTGACCCTA
TCTTGCATCTGACTTACAGAGCTTAGCTTCCGCTTCCGGCCCGGACAGAAACCGCCACGACCGCTGCACAAGCCCAGGGCTATATCCAGGAGG
CTACCGGTACGTTGGTGAACCTGACCTCGAGAAGTCTTCGACAGATGAACACAGATATCTGATGAGCAGAGTGGCCAGAAAGGTGAAGGACAA
GCGAGTCTGAAGCTGATCAGAGCCTACTGCAAGCTGGAGTAGTATGATGAGGCGGTAAGGTGACAGCCGAGGAAAGGCCACCTCAGGGTCCGCC
TCTGAGCCCTGCTGGCCAAACATCTGCTGGACGACCTGGACAAGGAACCTGGAAAACCGGGCCTGAAATTTGTAGATACGCCGATGACTGCAAC
ATCTACGTGAAAAGCTGCGGGCAGGCCAGAGAGTCAAGCAGAGCATCCAGAGATTCCTGGAAAAGACCCTGAAGCTGAAAGTGAATGAGGAAAAAG
TCTGGCGTGGACCTTGGAAAGAGAGCCTTCTGGCTTTTCTACCCCGAGAGAAAGGCCAGAATCCCGCTGGCCCTCGGAGATTCAGG
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GGCTACTTTAGACTGGTGAAACCCCTAGCGTCTTCAAACAATCGAGGGATGGATCAGACGAGACTGAGACTCTGCCAGTGGCTGCAGTGGAAGA
GAGTGGCGACAAGAATCAGGGAAGCTGAGAGCTCTGGGCTGAAAGAGACAGCTGTGATGGAATCGCCAACACCAGAAAGGGCGCCTGGGCGACC
ACCAAGACACCACAGCTGCACCAGGCCCCTGGGCAAGACCTAGTACTGGACCCCGGGGACTGAAATCCCTGACCAGAGATATTCGAGCTGAGACAG
GGC

Tel4c group II intron RT (1686 bp); Tel4c group II intron RT-truncation 1 (1506 bp): Tel4c group II intron RT-truncation 1 (1209 bp)

ATGGAAACCAGGCAGATGACAGTGGATCAGACCACCGGCGCGTGACCAACCAAACAGAGACAAGCTGGCACAGCATCAACTGGACC AAAGCCAAAC
AGAGAGGTTAAGAGACTGCAGGTGCGGATCGCTAAGGCCGTTAAAGAAGGCAGATGGGGCAAGGTGAAGGCCCTGCAATGGCTGCTGACCCACTCC
TTCTACGGCAAGGCTCTCGCCGTGAAACGGGTGACCGACAACAGCGGCTCTAGAACACCCGGCGTGGACGGCATCACCTGGAGCACCAGGAGCAG
AAAACCCAAAGCCATCAAAAAGCCTGAGAAGAAGAGGCTACAAGCCCAAGCCTCTGGCGAGAGTGTACATCCCTAAGGCCAAACGGCAAGCAGAGACT
CTGGGCATCCCCACCATGAAGGACCGGGCTATGCAGGCCCTGTACGCCCTGGCACTTGAACCTGTGGCCGAGACTACAGCCGACAGAAACAGCTACG
GATTCAGAAAGAGGCAGATGACCGCCGATGCCCCTGGCCGCTCTGCGCGTGGGCTCTGCGCAGAGTGAAGCCAAGAGCCGCGGACGACGTGCTGGACCGCCGAT
CTCTGGCTGTTTTGATAACATCAGCCACGAGTGGTGTCTGGCCAATACCCTCTGGACAAGGGAATTCGAGAAAGTGGTGAAGTCTGGTCTCTGTG
GGAAGCAGCAGCTCTCCCCACACAGCCGGCACCCCTCAGGGCGGCGTGATCTCCCTGTGCTGGCCAATATCACCTGGATGGCATGGAAGAGCT
GCTGGCTAAGCAGCTGCGGGGACAGAAGTGAACCTGATGGCGGTGGCCGATGATTTCTGTTGACCCGAAAAGATGAGGAAACCCCTGGAAGAAAGGC
CAGAAACCTGATCCAAGAGTTCCTGAAGGAAAGAGGCCTGACACTGTCTCTGAGAAGACAAAGATCGTGCACATCGAGGAAGGTTTTGACTTCTGT
GGATGGAACATCAGAAAGTACAACGGCGTCTGCTGATTAAGCCTGCCAAGAAGAACGTGAAGGCCCTTCCTGAAAAGATCCGGGATACCTGGCG
GAAGTGAAGCCGCACACAGGAGATCGTGATCGACACTCAACCCATCATCAGAGGATGGGCTAACTACCACAAGGGCCAAGTCAGCAAGGAA
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AGGCCAGCGCAGTATCCGACAGAGACTCCGACAGAGAGTCCGACAGGCTGCGGCGGCTTGCCTGTGTCGGCGGAAAATGAGCAAGGACGATGC
TGACAGACATCCACCACATCTGCCCCAAGCACAAGGCGGTTCCGACGACCTGGATAACCTGGTGTGATCCACGCCAACTGCCACAAGCAGGTGCA
CAGCCGGACGGCAGACTCTCGGAGCTGTGAAAGAGGGCCTG

Supplemental Data 2: codon usage M-MLV RT variants

IDT M-MLV RT

ACCTTAAACATAGAGGACGAATACCGACTCCACGAAACGAGCAAGGAACCTGATGTTTCTTTGGGAGTACGTGGTTGTCCGACTTTCCGCAAGCCT
GGGCGAAACGGGGGATGGGTCTCGCAGTACAGACGGCTCCTCTTATCATTCCTTTGAAAGCGACTCAACGCCAGTGTCAATCAAACAATACCC
AATGAGTCAAGAGCAAGGCTCGGAATAAAGCCCCATATACAAAGGCTCTCGATCAGGGGATCTTGGTACCATGCCAAAGCCATGGAATACGCCCT
CTCTCCCGTCAAGAAACCGGACAGAACGATTATAGACCGTTCACCGCTCCTCGGCAAGTCAATAAGCGGTTGAGGATATACCGACAGACTAC
CTAACCCTACAATCTGTCTGTGCTGGCCTGCCACCGTACACCAATGGTATACCCTATTGGACCTGAAAGATGCCTTTTTTCTGCCTGAGACTGCACCCCA
CCTCCAAACCTATGTTTGCATTTCAGTGGAGGGATCCAGAAATGGGATTGAGGCAATGACTTGGACCAAGGCTCCCCCAAGGTTTTAAAATTTCA
CCAACCTTTTTAATGAGGGCTGCACAGAGACTTGGCCGCTTGAAGTTCACACCCCGACTTCTTTTGGCAATTTCTTTTGGCAGTTCGCTGCTC
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CCGGAAGACACTAGCCACAGTGGGAGTTCTGGGTAAGACCGGATTTGGCAGCTTTTATACCGGTTTGGCGAAAATGGCAGCCCCCTTACC
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GAATGACGCACTACCAAGCCGCTGCTCTGATAGTATAGAGTACAATTTGGACCTGCTGCTGCAATTGAAACCCAGCAGACTTTCTCCGTTGCCAGAG
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GCGGGTACTTACAGCAGCGGGCAGAAATGATCGCCCTACCAAGGCGTTAAGATGGCCGAAAGGTAAGAAGCTCAACCTTTACAGGACAGTAGGT
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ATGGTGAACAAAGCTGCCGGAAGGCCGCAATCACTGAAACCCAGATACCTAACCCCTTCTATCGAAGACAGAGCCCT

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ACCTGAAACATGAAAGTGAAGTACAGACTGCACGAGACATCTAAGGAACCTGATGTGTCCCTGGGTTCTACATGGCTGAGCGACTTCCCTCAGGCCT
GGGCTGAAACCGGGGATGGGCTGGTGTGCGGAGGCTCCTCTGATCATCCCTCTGAAGGCCACTCCACCCTGTGTCCATCAAGCAGTACCC
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TCTAGATACGCTTTGCCACAGCCACATCCAGGCGAGATCTACAGACGAGAGGATGGCTAACCTCTGAGGGCAAGGAAATCAAAAACAAGGAT

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ACCCTGAACATCGAGGACGAGTACCAGGCTGCACGAGACAAGCAAGAACCAGATGTGTCCCTGGGCAGCACCCTGGCTGTCTGATTTTCCACAGGCCT
GGGCCGAGACAGGCCGAATGGGACTTGCTGTTAGACAGGCCCTCTGTATCATCCCTCTGAAGGCCACAAAGCACCCCTGTGTCCATCAAGCAGTACCC
CATGAGCCAAGAGGCCCGCTGGGAATCAAGCCCCACATTCAGAGACTGCTGGACCAGGGCATCTGGTGCCTTGTCAAGACCCTTGGAAATACCCCT
CTGTGCCCCGTGAAGAAGCCCGCACAACGATTACAGACCCGTGAGAGCTGCGGGGAAGTGAACAAGAGAGTGAAGATATCACCCACCCTG
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GCCCCACTGTTCACGAGGCCCTGCACAGAGATCTGGCCGACTTCAGAATTACAGCCCCGACCTGATCCTGCTGCAGTACGTTGACGATCTGTGT
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CAGATCTGCCAGAAAAGTGAAGTACCTGGGCTACCTGTGAAAGAGGGCCAGCGTTGGCTGACCAGGCCAGAAAAGAACCCGTGATGGGCCAG
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ACACTGAATATCGAGGACGAGTACAGACTGCACGAAACCAGCAAGGAGCCCGATGTGTCTCTGGGCTCCACATGGCTGAGCGATTTTCCCAGCTT
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ACTCTTAACATCGAGGACGAATACCCTTACGAAACTCTAAGGAAACCGGATCAGCCTGGCTCAACCTGGCTCTCAGCCCTCCCAAGCTTG
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ACCCTTAATATTGAAGTATGATTCGGCTACATGAAACATCAAAGAGCCAGATGTTTCTTTGGGTCCACATGGCTGCTGATTTCCCTCAGGCTTG
GGCGAAACCAGGGGATGGGCTGCGAGTTCGCCAGGCTCCTCTGATTATACCTCTGAAGGCAACCTTACCCTGTTGCATAAAACAGTACCC
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GGCCGCCACATCTGAGCTGGACTGCCAACAGGGTACACGGGCGCTGTTACAAACCCTAGGCAACCTCGGTTATCGGGCTTCGGCTAAGAAAGCCAG
ATTTGCCAGAAACAGGTCAAGTATCTGGGCTATCTTTTGAAGAGGGTACAGAGATGGCTGACTGAGGCCAGAAAAGAGACTGTGATGGGGCAGCCTA
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CCTCTACCAAACCGGCACTCTGTTCAATTGGGGCCAGACCAACAGAAGGCCTATCAGGAAATCAAGCAGGCTCTTTTAACTGCCCCAGCCCTGG
GCTTGCCAGATTGACTAAGCCCTTTGAGCTCTTTGTGGACAGGACAGGGTCCAGTTCGGACCTGTGGTAGCCCTGAATCCGGCTACACTGCTCCACTGCT
GCCGGTGGCCTACCTGAGCAAAAAGCTGGACCCAGTAGCAGCTGGGTGGCCCCCTGTTTTCGGGATGGTTCGACGCTATTGCCGTGTGACAAAGGAT
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CCAGGATGACTACTATCAGGCCTTGGCTTTTGGACACGGACAGGGTCCAGTTCGGACCTGTGGTAGCCCTGAATCCGGCTACACTGCTCCACTGCT
GAGGAAGGGCTGCAACAACTGCCTTGATATCTGGCCGAAGCCACGGAACCCGACCCGATCTTACGGACCAGCCGCTCCAGACGCGGACCATA
CCTGGTACACGGATGGAAGCAGCTCTTACAAGAGGGCCAGCTAAGGCGGGAGCTGCGGTGACCACCGAAACAGAGGGTGAATCTGGGCTAAAGCC
TGCCAGCCGGCAGCTCCGCTCAGCTGATGATTGACACACAGAGTGGTACACCGTGGACTGAAAGATGGCAGAAAGGTAAGAAGACTGATGTTTGGCAGT
CCGTTATGCTTTGCTACTGCTCATATCCATGGAGAGATATACAGAAGGCGTGGGTGGCTACATCAGAAGGCAAGGAGATTAATAAAGACGAG
ATCTTGGCGTGTCTTAAAGCCCTCTTTCTGCCAAAAGGCTTAGCATTATTCATTGTCCAGGACATCAAAAGGGACACAGCCGCGAGGCTAGAGGCAA
CAGGATGGCTGACCAAGCGGCCGAAAGGCAGCCATCACAGAGACTCCAGACACATCTACCCCTCTCATAGAAAATAGTAGCCCC

Supplemental Data 3: Truncated M-MLV RT^{CO}

RT^{CO}-ΔR (1560 bp)

ACCTGAAACATCGAAGATGAGTACAGACTGCACGAGACATCTAAGGAACCTGATGTGTCCTGGGTTCTACATGGCTGAGCGACTTCCCTCAGGCCT
GGGCTGAAACCGCGCATGGGCTGGCTGTGCGGCAGGCTCCTCTGATCATCCCTCTGAAGGCCACTCCACCCCTGTGTCCATCAAGCAGTACCCC
ATGAGCCAGGAGGCCAGACTGGGCATCAAGCCCCACATCCAGCGGCTGCTCGACCAGGGAATCTTGGTCCCTGCCAGAGCCCTGGAACACCCAC
TTTTACCTGTGAAAAAGCCCGCACTAACGACTACCGGCTGTGACAGGACCTGAGAGAAGTGAACAAGCGGGTTGAGGACATCCACCCCTACAGTGCC
TAACCCCTACAACCTGCTGTCTGGCCTGCCACCGAGCCACAGTGGTACACCGTGGACTGAAAGAGCGCCTTCTTGTCTGAGACTCCATCCTA
CCAGCCAGCCACTGTTTCGCTTCGAGTGGCGGGACCTGAGATGGGCATCAGCGGCCAGCTGACATGGACCCGGCTGCCACAGGGCTTCAAGAACAG
CCCTACACTGTTCAACGAGGCCCTGCACAGAGATCTGGCTGACTTCAAGATCCAGCATCTGACCTGATCTGCTGCAGTACGTGGACGACTTGTCTG
TGGCCGCTACATCTGAATGGATTGCCAGCAAGGCACAGAGCTCTGCTCCAGACCCCTGGGCAACCTGGGGTATAGGGCCAGCCAAAGAAGGCCA
GATCTGCCAGAAGCAAGTGAAGTACCTGGGATACCTGCTGAAGGAAGGCCAGAGATGGCTGACCGAGGCGCGGAAGGAAACAGTGTGGGCCAGCC
TACCCCTAAGACCCCGAGACAGCTGCGAGAATCTCTGGGCAAGGCTGGCTTTTGCAGACTTTCATCCCGGCTTCGCTGAGATGGCCGCTCCTCTGT
ACCCACTGACCAAGCCCGTACCTGTTCAATTGGGGCCCGATCAGCAGAAAGCCCTACCAGGAGATTAAGCAGGCCCTGCTGACCCGCCCTGCCCT
CGGGCTGCTGATCTGACAAAACCTTTCGAGCTGTTTGTGACGAGAGAAGCAGGGCTACGCCAAGGGCGTGTGACACAGAACTGGGACCTTGAGAG
AGACCTGTGGCTTATCTCAGCAAGAAGCTGGACCCCGTGGCCGCGGATGGCTCCATGCTGAGGATGGTTCGCCGCTATCGCCGTGTGACCAAGG
ACGCCGGCAACTGACCATGGGACAACTCTGGTGTCTGGCCCTCACGCCGCTGAGGCCCTGGTGAAGCAGCCTCTGACAGATGGTGTGAGCAA
CGCCAGAATGACCCACTACCAGGCCCTGCTGCTGGATACCGATAGAGTGCAGTTCGGCCCTGTGGTGGCTTGAATCTGCCACCTGCTCCACTTC
CCGAGGAAGGCTGCAGCAACTGCTGGACATCTTGGCAGAAGCCACGGCACAAGACCTGACTTAACAGACCAGCCTTGTCTGATGCGGACCA
CACC

RT^{CO}-ΔR-m (1410 bp)

CTGGGTTCTACATGGCTGAGCGACTTCCCTCAGGCCTGGGCTGAAACCGCGGCATGGGCTGGCTGTGCGGCAGGCTCCTCTGATCATCCCTTGAA
GGCCACCTCCACCCCTGTGTCCATCAAGCAGTACCCCATGAGCCAGGAGGCCAGACTGGGCATCAAGCCCCACATCCAGCGGCTGCTCGACCAGGGA
ATCTGTGTCCTGCCAGAGCCCTGGAACACCCCACTTTTACCCTGTGAAAAAGCCCGGCACTAACGACTACCGGCTGTGACGACCTGAGAGAAG
TGAACAAGCGGGTTGAGGACATCCACCCTACAGTGCCTAACCCCTACAACCTGCTGTCTGGCTGCCACCGAGCCACCAGTGGTACACCGTGTGGA
CCTGAAAGACGCCTTCTTCTGTCTGAGACTCCATCTACCAGCCAGCCACTGTTTCGCTTCGAGTGGCGGGACCTGAGATGGGCATCAGCGGCCAGC
TGACATGGACCCGGCTGCCACAGGGCTTCAAGAACAGCCCTACACTGTTCAACGAGGCCCTGCACAGAGATCTGGCTGACTTCAAGATCCAGCATCC
TGACCTGATCTGCTGCAGTACGTGGACGACTTGTGCTGGCCGCTACATCTGAATGGATTGCCAGCAAGGCACCAGAGCTTGTCTCCAGACCCTGG
GCAACCTGGGGTATAGGGCCAGCGCAAGAAGGCCAGATCTGCCAGAAGCAAGTGAAGTACCTGGGATACCTGCTGAAGGAAGGCCAGAGATGGC
TGACCGAGGCGCGAAGGAACAGTGTATGGGCCAGCCTACCCTAAGACCCCGAGCAGCTGCGAGAATTCCTGGGCAAGGCTGGCTTTTTCAGACT
GTTTCATCCCGGCTTCGCTGAGATGGCCGCTCCTCTGTACCCACTGACCAAGCCCGGTACCCTGTTCAATTGGGGCCCGATCAGCAGAAAGCCTACC
AGGAGATTAAGCAGGCCCTGCTGACCGCCCTGCCCTGGGCTGCCTGATCTGACAAAACCTTTTCGAGCTGTTTGTGACGAGAGAAGCAGGGCTACGC
CAAGGGCGTGTGACACAGAACTGGGACCTGGAGAAGACCTGTGGCTTATCTCAGCAAGAAGCTGGACCCCGTGGCCCGGATGGCCTCCATGT
CTGAGGATGGTTCGCCGCTATCGCCGTGTGACCAAGGACCGCCGCAAACTGACCATGGGACAACCTCTGGTGTCTGGCCCTCACGCCGCTGAGG
CCCTGGTGAAGCAGCCTCTGACAGATGGCTGAGCAACGCCAGAATGACCCACTACCAGGCCCTGTGCTGGATACCGATAGAGTGCAGTTCGGCC
TGTGGTGGCTTGAATCTGCCACCTGCTCCACTTCCGAGGAAGGC