

Supplementary Information

No evidence for epitranscriptomic m⁵C modification of SARS-CoV-2, HIV and MLV viral RNA

Anming Huang¹, Lydia Rieper², Dietmar Rieder³, Janine Kimpel² and Alexandra Lusser^{1*}

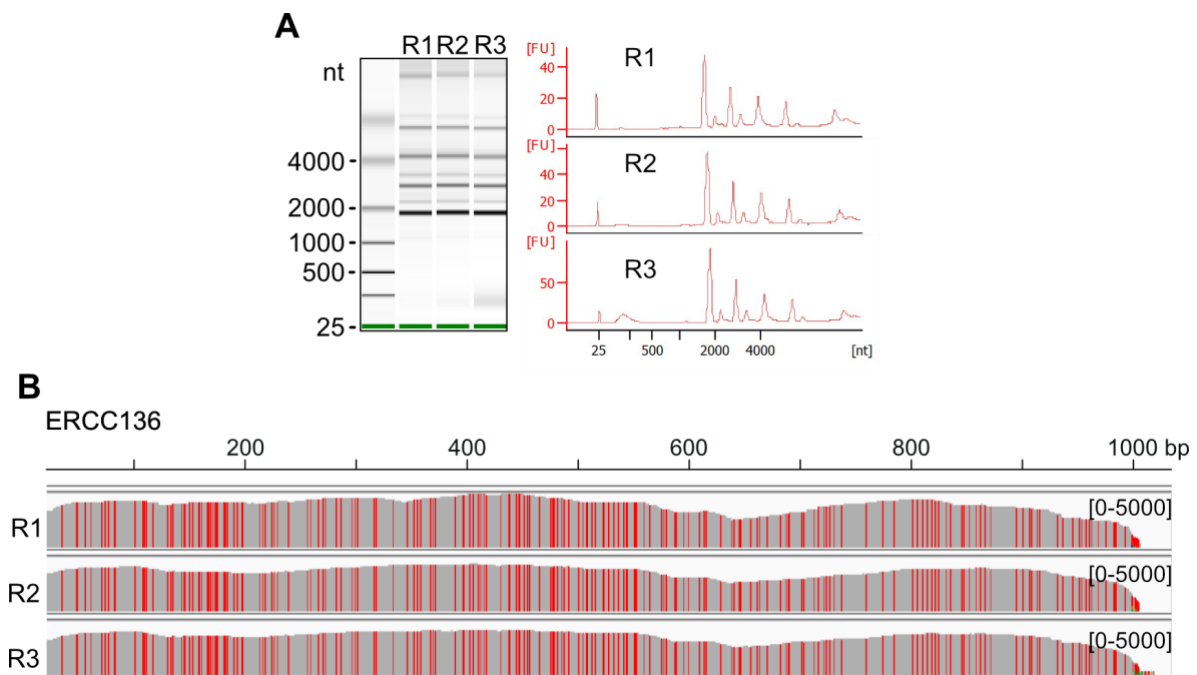
¹ Institute of Molecular Biology, Biocenter, Medical University of Innsbruck, Innsbruck, 6020, Austria

² Institute of Virology, Department of Hygiene, Microbiology and Public Health, Medical University of Innsbruck, Innsbruck, 6020, Austria

³ Institute of Bioinformatics, Biocenter, Medical University of Innsbruck, Innsbruck, 6020, Austria

Supplemental Table S2. Primers for amplification of BS-treated RNA

	Primer sequence
<i>SARS-CoV-2</i>	
Sars-Cov-2_BS1_FW	5' TGTTAGAATGGTTGGCAATGG
Sars-Cov-2_BS1_RV	5' CCAAACTTCTTAAAAACCTCA
Sars-Cov-2_BS2_FW	5' AAATTTAAGGAAATTTGGGGA
Sars-Cov-2_BS2_RV	5' TATAAATCAACCACATTCCC
<i>HIV</i>	
HIV_BS1_FW	5' ATGAAAGATTGTATTGAGAGA
HIV_BS1_RV	5' TTATTATCTCTTCCCCAACCT
HIV_BS2_FW	5' TTTAAAAGAAAAGGGGGGA
HIV_BS2_RV	5' TTAACCTCTTCTACCTTATCT
<i>MLV</i>	
MLV_BS1_FW	5' ATTTGGTGGATTTGTGGTGG
MLV_BS1_RV	5' AACCACATATCCCTCCTCTA
MLV_BS2_FW	5' GTGGTTTTTTAATATAAGTTG
MLV_BS2_RV	5' CAACTAAATAATAAAACCCTC
<i>Human tRNA_{Asp}</i>	
tRNAAsp_FW	5' TTGTTAGTATAGTGGTGAGT
tRNAAsp_RV	5' CCATCAAAAAATCAAACCCC
<i>Mouse tRNA_{Asp}</i>	
mtRNAAsp_FW	5'TGTTAGTATAGTGGTGAGTAT
mtRNAAsp_RV	5'CTCCCCATCAAAAAATTA
<i>ERCC</i>	
ERCC136_FW	5' TGTGAATTTGGTAAGTTTG
ERCC136_RV	5' TCTAACATCTTCTCTTCATC



Supplemental Figure S1. (A) The quality of RNA isolated from SARS-CoV-2-infected Vero E6-TMPRSS2-ACE2 cells after three rounds of poly(A) enrichment was assessed by Agilent Bioanalyzer. Gel-like image (left) and electropherograms (right) of three biological replicates (R1, R2, R3) are shown. (B) Example IGV tracks of ERCC136 RNA show sufficient read coverage in all replicates. Red lines correspond to mismatches to the reference genome due to C-to-U conversion by bisulfite treatment.

HIV.1 (pNL4-3)	ATGAAAGATTGTA	CTGAGAGACAGGCTAATTTTTAGGGGAAGATCTGG	CC	CTTCCCACAAG	60
HIV.1 (D117/II)	ATGAAAGATTGTA	CTGAGAGACAGGCTAATTTTTAGGGGAAGATCTGG	CC	CTTCCCACAAG	60
Colony3	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony22	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony21	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony20	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony19	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony18	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony17	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony16	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony14	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony13	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony12	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony11	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony10	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony9	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony8	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony7	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony6	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony5	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony4	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony2	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony1	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATTGGT	TTTTT	TATAAG	60
Colony15	ATGAAAGATTGTA	CTGAGAGATAGGTTAATTTTTAGGGGAAGATCTGGT	TTTTT	TATAAG	60
	*****	*****	***	*****	***
HIV.1 (pNL4-3)	GGAAGG	CCAGGGAATTTTCTTCAGAGCAGACCAGACCCACAGCCCCACCAGAAGAGAGC	120		
HIV.1 (D117/II)	GGAAGG	CCAGGGAATTTTCTTCAGAGCAGACCAGACCCACAGCCCCACCAGAAGAGAGC	120		
Colony3	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony22	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony21	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony20	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony19	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony18	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony17	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony16	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony14	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony13	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony12	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony11	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony10	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony9	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony8	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony7	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony6	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony5	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony4	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony2	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony1	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
Colony15	GGAAGGTTAGGGG	ATTTTCTTCAGAGTAGATTAGAGTTAATAGTTTATTAGAAGAGAGT	120		
	*****	*****	***	*****	***

Supplemental Figure S2. Sanger sequencing results of region 2056-2174 in HIV D117/II RNA. Multiple sequence alignment of individual clones after bisulfite treatment with the corresponding sequences in HIV NL4-3 and HIV D117/II. Cs identified as m⁵C by Cristinelli et al. (2021) are marked in red. All clones show complete C-to-U conversion.