

Nano-DMS-MaP allows isoform-specific RNA structure determination

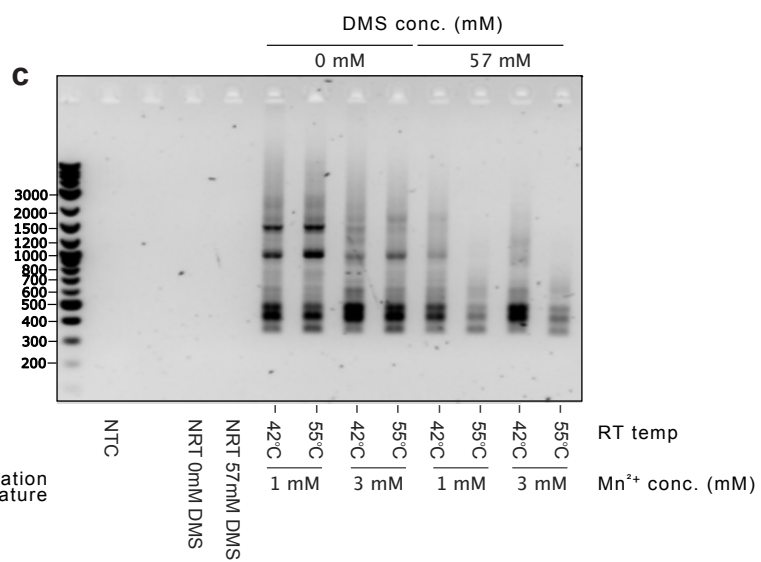
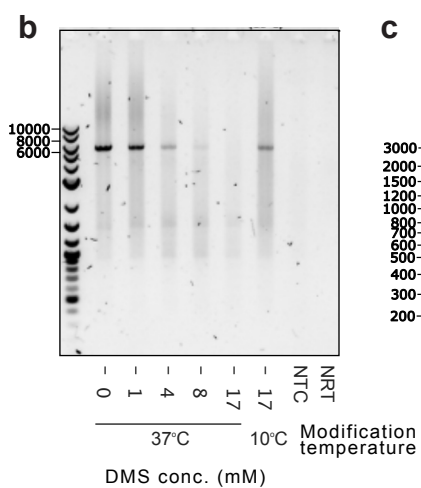
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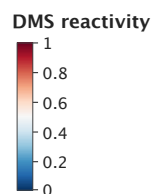
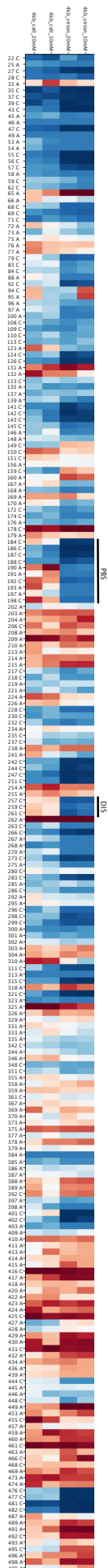
Suppl. Figure 1: Optimization of reverse transcription conditions

(a) RT-PCR of a ~500 nt fragment of the HIV-1 unspliced RNA containing the highly structured 5'UTR from HIV-1 expressing cells treated with different concentrations of DMS. (b) Optimization of a 5.5 kb RT-PCR from HIV-1 expressing cells by varying DMS concentrations and DMS modification temperature. (c) Optimization of a RT-PCR of a complex splicing mixture from HIV-1 expressing cells by varying DMS concentration, Mn^{2+} concentration, and reverse transcription temperature. DNA ladder was 1kb plus (NEB).



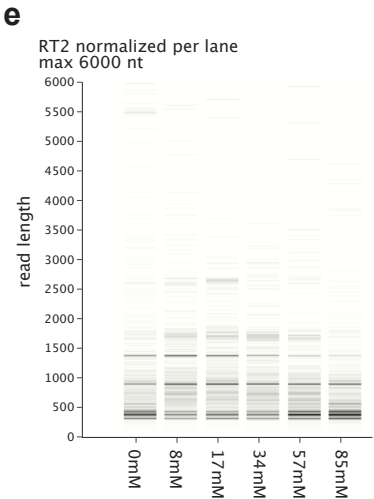
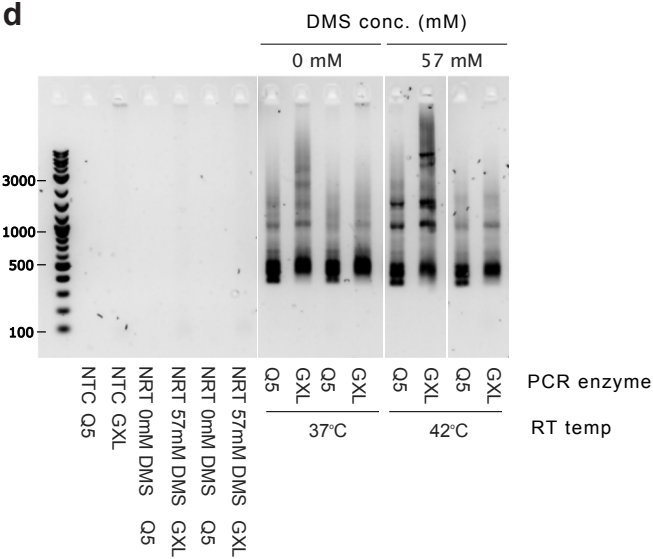
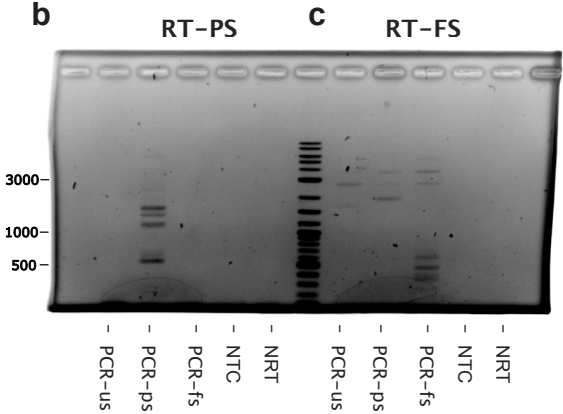
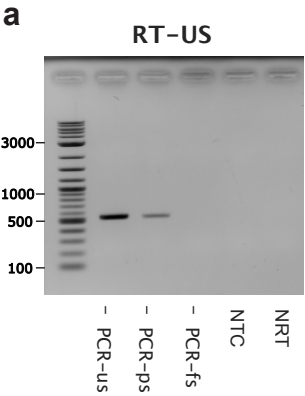
Suppl. Figure 2: Heat map of 5'UTR DMS reactivities for cell and virion samples

Heat map of DMS reactivities for A and C residues for the unspliced HIV-1 RNA found in cells and virions at two DMS concentrations (10 mM and 20 mM). DMS reactivities shown for each DMS concentration is a mean of two independent experiments. The primer binding site (PBS) which binds a cellular tRNA in virions is highlighted. The dimerization initiation site (DIS) which forms an inter-molecular interaction is highlighted.



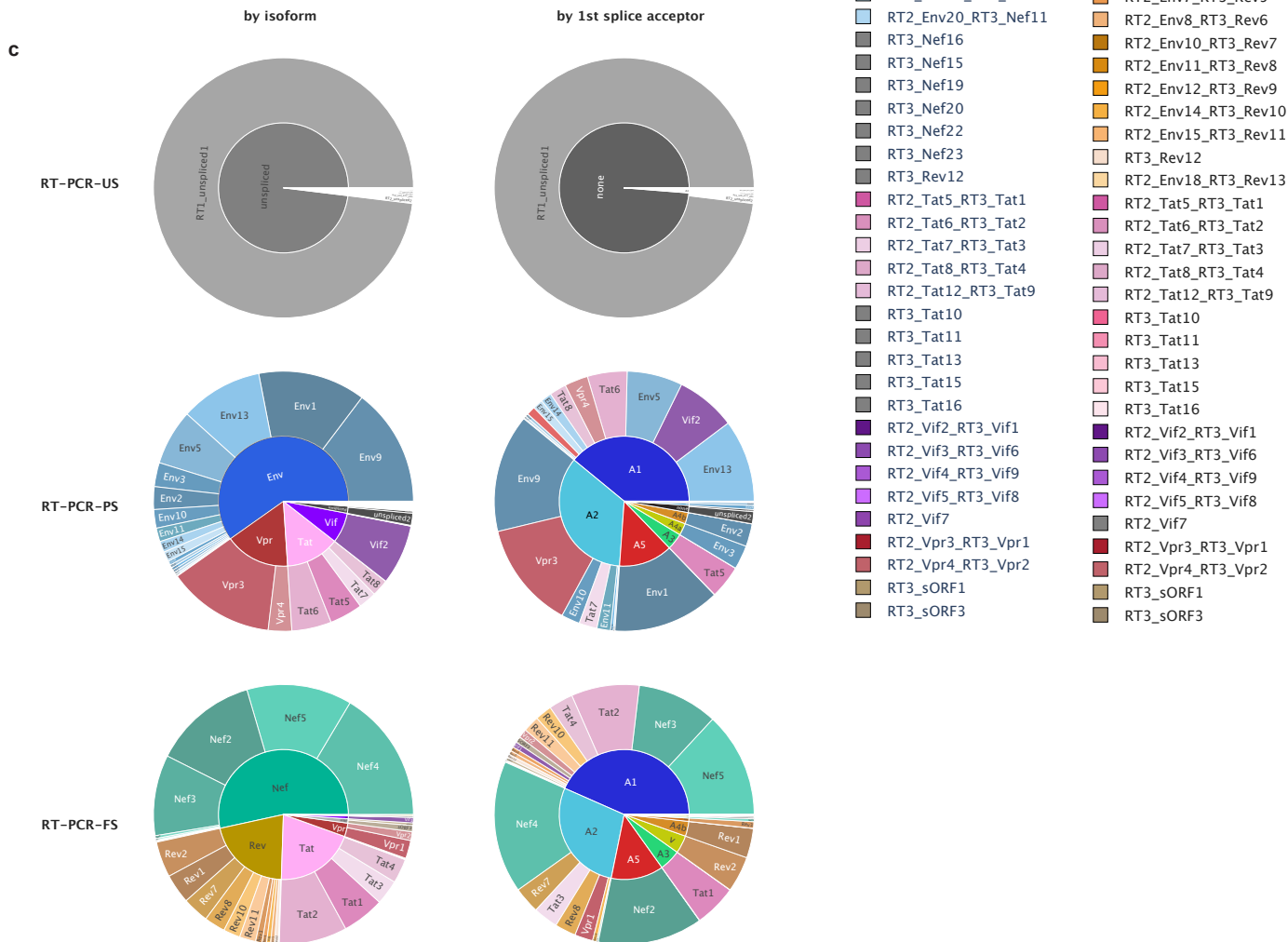
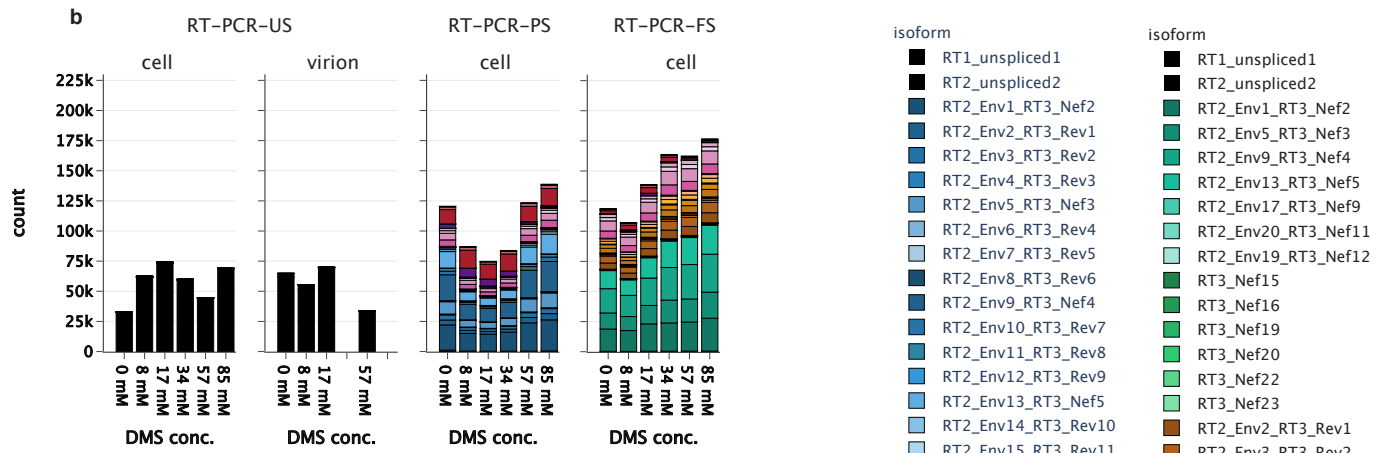
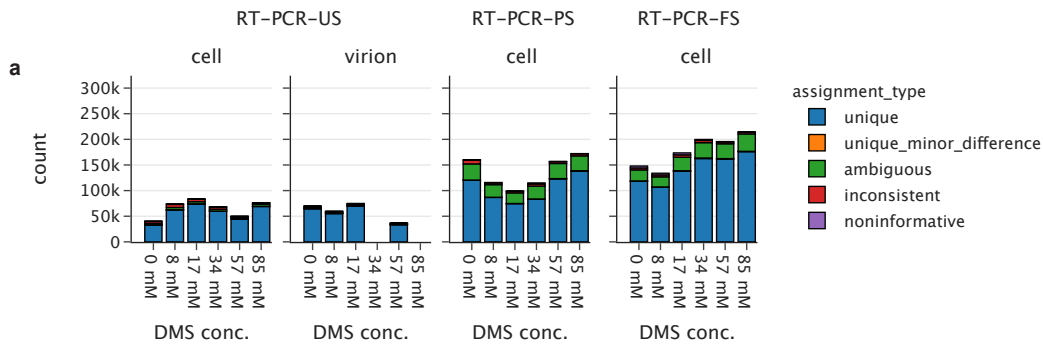
Suppl. Figure 3: Specific RT-PCR for the HIV-1 splicing landscape

(a) RT-PCR-US specifically amplifies unspliced genomic RNA (b) RT-PCR-SS specifically amplifies a population of single spliced RNAs. (c) RT-PCR-FS specifically amplifies a population of fully spliced RNAs. (d) Optimization of the RT-PCR-FS with DNA polymerase Q5 and PrimeSTAR GXL at two different reverse transcription temperatures. DNA ladder for all gels was 1kb plus (NEB). (e) Uncropped virtual gel showing diverse species amplified at each DMS concentration for RT-PCR-PS sample.



Suppl. Figure 4: Read counts per assignment and isoform

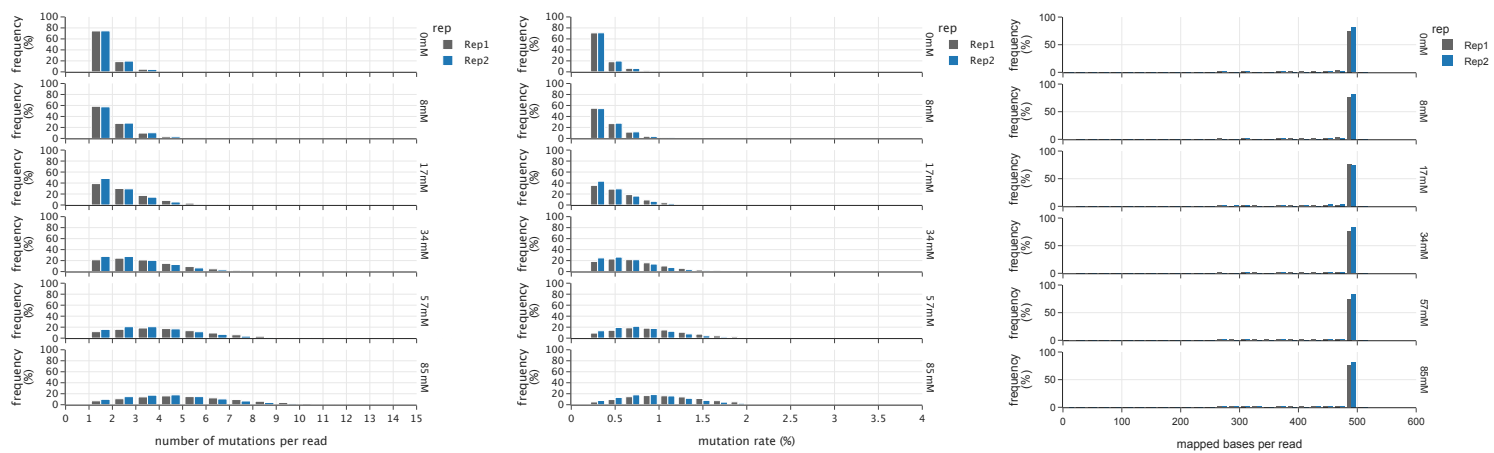
(a) Representative data from a single nanopore sequencing run showing read count per assignment IsoQuant type for RT-PCR-US, RT-PCR-PS and RT-PCR-FS. “Unique” are sequencing reads mapping uniquely to a transcript isoform. “Unique minor differences” reads map to a single isoforms, but with alignment artifacts. Ambiguous reads are compatible with matching to more than one transcript. Inconsistent reads map with non-intronic inconsistencies (e.g., alternative transcription or polyadenylation sites). Non-informative reads are unmapped reads. (b) Representative data from a single nanopore sequencing run showing read counts per isoform assignment for RT-PCR-US, RT-PCR-PS and RT-PCR-FS as determined by IsoQuant. (c) Sunburst plots showing relative expression of each transcript grouped by isoform (left) or first acceptor site (right).



Suppl. Figure 5: Mutation distribution in nanopore and Illumina datasets

Histograms of per read mutation count distributions (left), per read mutation percentages (middle) and per read aligned read length (right) as saved in RNA-Framework's mutation map (mm) files shown for **(a)** nanopore and **(b)** Illumina datasets obtained from matched samples of the unspliced (US) HIV-1 RNA probed at different DMS concentrations (rows). Data for independent experimental replicates are shown separately (blue and grey bars). Reads with 0 mutations are excluded from this analysis.

a Nanopore



b Illumina

