

Supporting information for:

Femtomole SHAPE reveals regulatory structures in the authentic XMRV RNA genome

Jacob K. Grohman^a, Sumith Kottegoda^a, Robert J. Gorelick^b, Nancy L. Allbritton^{a,c} and Kevin M. Weeks^{a,*}

^a Department of Chemistry, University of North Carolina, Chapel Hill 27599-3290

^b AIDS and Cancer Virus Program, Science Applications International Corporation-Frederick, Inc., National Cancer Institute-Frederick, Frederick, MD 21702-1201

^c Department of Biomedical Engineering, University of North Carolina, Chapel Hill, NC 27599 and North Carolina State University, Raleigh, NC 27695

*correspondence: weeks@unc.edu

Supporting Figures 1–5

Supporting Figure 1. Instrument limit of detection. Electropherograms of free 5-FAM (green) and 6-JOE (yellow) fluorophores shown at their 3:1 signal-to-noise detection limits. Note that the x-axis scale is 10-fold larger in the lower panel.

Supporting Figure 2. Precision and accuracy of high-sensitivity capillary electrophoresis.

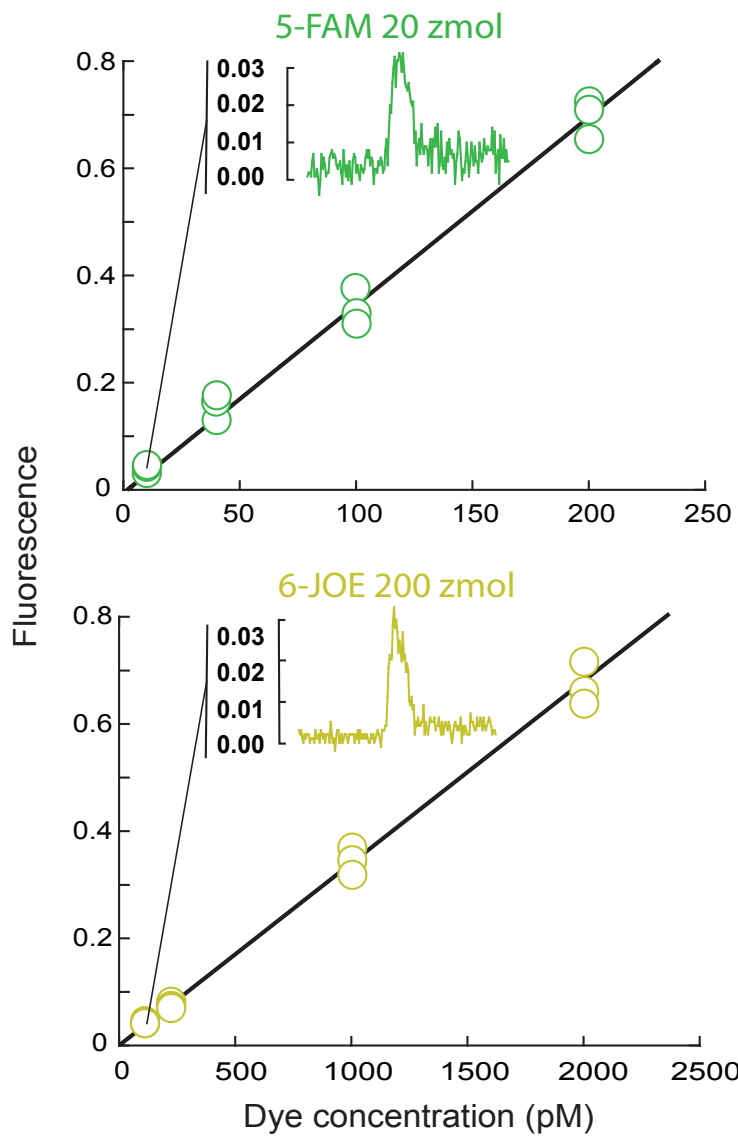
(A) Mean and standard deviations for three dideoxy GTP (ddGTP) sequencing reactions, performed using 1 fmol RNA. Error bars are given for every data point. Standard deviations were less than 5% of measured intensities throughout the trace. (B) Electropherograms for SHAPE reactions performed on the MuLV monomer using 5 mm 1M7 and detected using either conventional capillary electrophoresis at 1 pmol RNA (blue) or high-sensitivity capillary electrophoresis at 1 fmol input RNA (red). (C) Correlation of raw fluorescence intensities for conventional versus custom capillary electrophoresis.

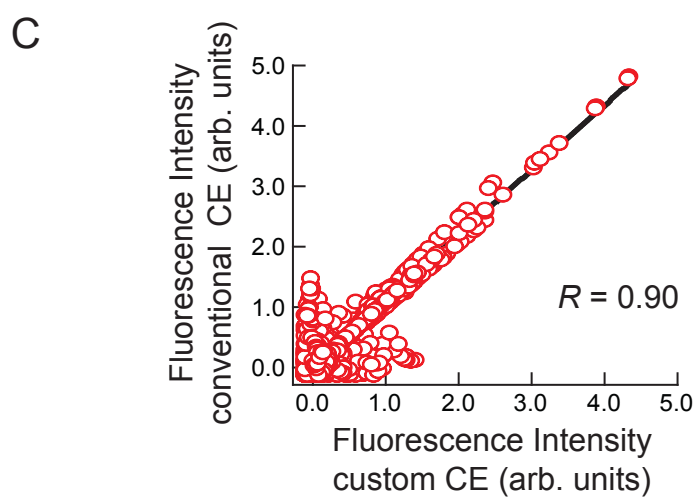
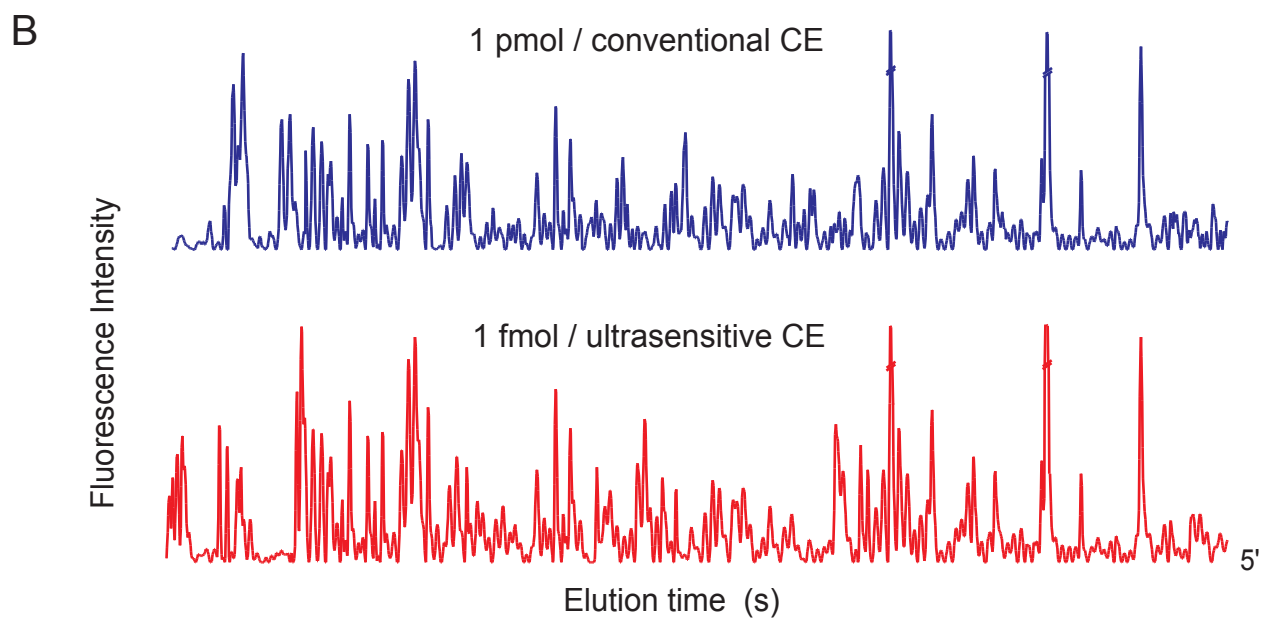
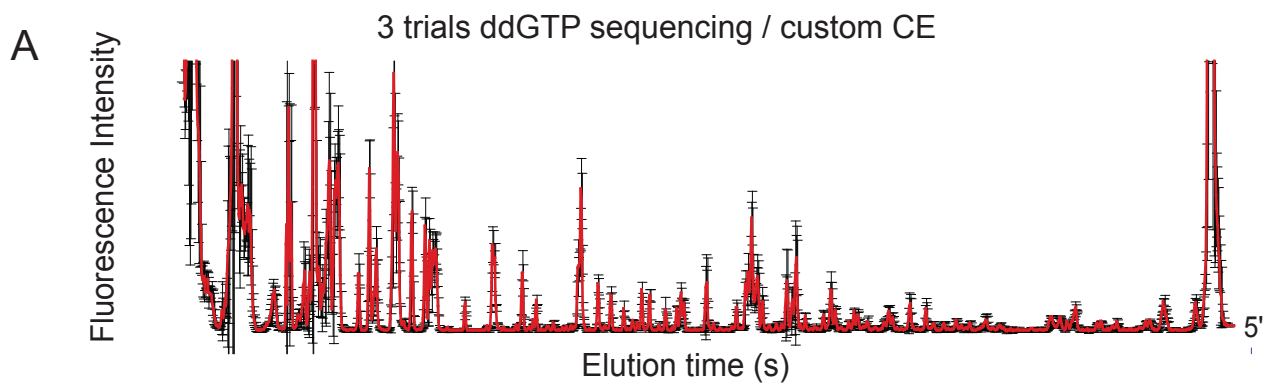
Supporting Figure 3. Comparison of XMRV and MuLV packaging domain sequences.

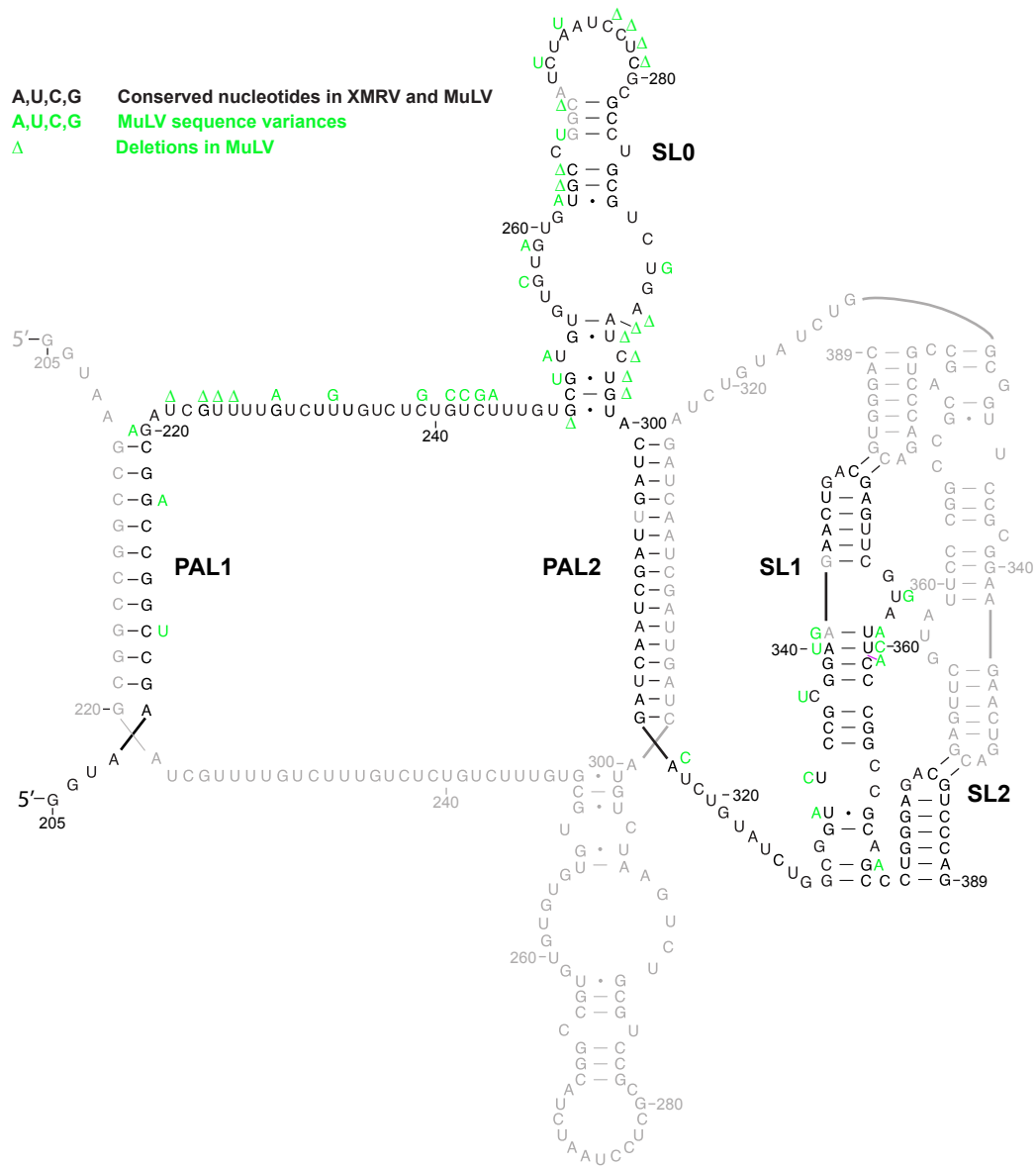
Sequence and secondary structure model of the XMRV packaging domain. The second strand is shown in gray. Nucleotides that differ in MuLV compared to XMRV are in green; deletions relative to MuLV are shown as green triangles.

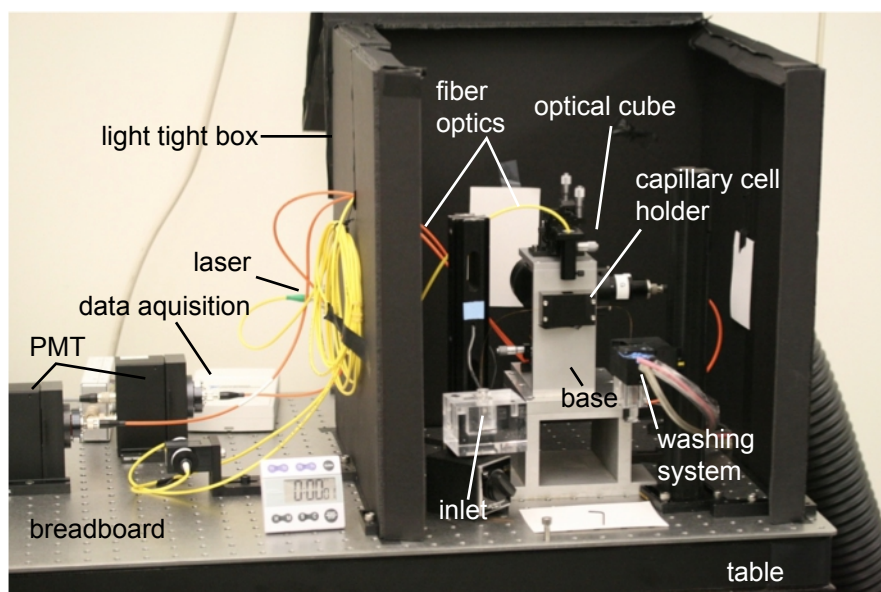
Supporting Figure 4. High-sensitivity capillary electrophoresis and key instrument components. Image of the instrument with major components labeled.


Supporting Figure 5. XMRV SHAPE data with forced folding as a monomer. Secondary structure model for the XMRV genomic RNA in a monomer state. Experimental SHAPE data from Figure 5A are superimposed as nucleotide colors. Regions where the SHAPE data are not compatible with the monomeric structural model are boxed.










 Regions where SHAPE data disagree with SHAPE-directed secondary structure prediction, assuming formation of a monomer

