RNase-Resistant Virus-Like Particles Containing Long Chimeric RNA Sequences Produced by Two-Plasmid Coexpression System[▾]

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ABSTRACT

RNase-resistant, noninfectious virus-like particles containing exogenous RNA sequences (armored RNA) are good candidates as RNA controls and standards in RNA virus detection. However, the length of RNA packaged in the virus-like particles with high efficiency is usually less than 500 bases. In this study, we describe a method for producing armored L-RNA. Armored L-RNA is a complex of MS2 bacteriophage coat protein and RNA produced in *Escherichia coli* by the induction of a two-plasmid coexpression system in which the coat protein and maturase are expressed from one plasmid and the target RNA sequence with modified MS2 stem-loop (pac site) is transcribed from another plasmid. A 3V armored L-RNA of 2,248 bases containing six gene fragments—hepatitis C virus, severe acute respiratory syndrome coronavirus (SARS-CoV1, SARS-CoV2, and SARS-CoV3), avian influenza virus matrix gene (M300), and H5N1 avian influenza virus (HA300)—was successfully expressed by the two-plasmid coexpression system and was demonstrated to have all of the characteristics of armored RNA. We evaluated the 3V armored L-RNA as a calibrator for multiple virus assays. We used the WHO International Standard for HCV RNA (NIBSC 96/790) to calibrate the chimeric armored L-RNA, which was diluted by 10-fold serial dilutions to obtain samples containing 10^6 to 10^2 copies. In conclusion, the approach we used for armored L-RNA preparation is practical and could reduce the labor and cost of quality control in multiplex RNA virus assays. Furthermore, we can assign the chimeric armored RNA with an international unit for quantitative detection.

FOOTNOTES

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