Use of encapsulated RNA as a stable and quantitative positive control for infectious disease detection, wastewater surveillance, and more

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Introduction

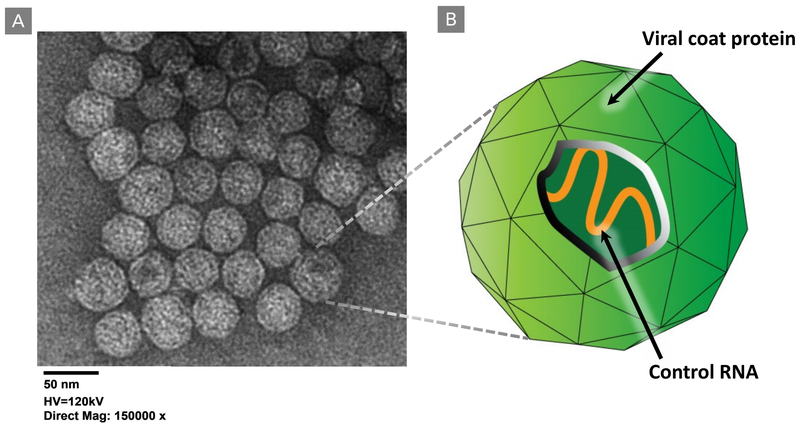
Why encapsulated RNA (en-RNA)?

Every nucleic acid detection assay requires a reliable, easily quantified positive control. In the case of ribonucleic acid (RNA) testing, stability in the presence of ribonucleases (RNases) is critical. Failure to provide such a control can lead to false negatives and failed experiments. Currently, positive RNA controls often come from in vitro transcription (IVT), inactivated pathogen, and plasmid DNA. Recently, en-RNA has emerged as a leading alternative. Ongoing efforts seek to improve the ease of creating en-RNA controls.[1] [2]

A new system for production of control RNA encapsulated in phage-like particles

Here, we present a single plasmid, double expression system for encapsulating any control RNA sequence within MS2 phage virus coat protein. This technique will produce highly stable, non-infectious capsules of RNA. These capsules make ideal calibration standards or internal assay controls because they are resistant to RNase digestion. Using common extraction methods, it is possible to extract encapsulated template RNA for use as quantification or size standards. Further, en-RNA can be used as a transient gene expression system.

Because our encapsulation technique accommodates RNA sequences of variable length, it is an ideal way to create controls for a variety of applications.



En-RNA particles are highly stable, non-infectious positive controls for a variety of applications. By Transmission Electron Microscopy (TEM), SARS-CoV-2 (SC2)-encapsulated RNA particles **(Panel A)** were found to be intact in the final storage buffer. The average size of particles was calculated to be very close to the size of natural virus (~27 nm). Most of the individual capsules were observed to have icosahedral symmetry, which matched our design for the encapsulated RNA viral particle **(Panel B).**

Methods

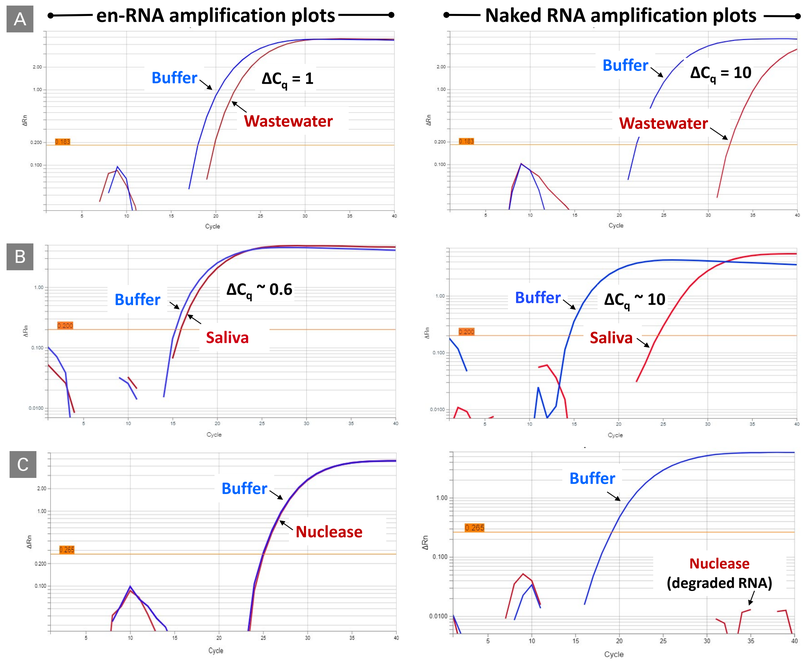
Our technology, a single plasmid, double expression system, produces phage-like particles that are very stable, nuclease resistant, and encapsulate high-quality RNA. Since encapsulated RNA mimics natural phage virus particles, they serve as ideal controls for processes like RNA extraction, reverse transcription, PCR amplification, and detection in the areas of infectious disease, wastewater surveillance, NGS workflows and more.

An *E. coli* system was used to express and assemble the MS2 phage-like viral particles. The resulting capsids can contain any RNA sequence of interest, ranging from 150 bp to 4 kb. Highly pure encapsulated RNA viral particles were produced by affinity purification using liquid chromatography.

Different RNA extraction methods, such as commercial kits, phenol-chloroform extraction, and heat lysis, were compared for the extraction efficiency. We found very similar efficiency with all three methods (data not shown).

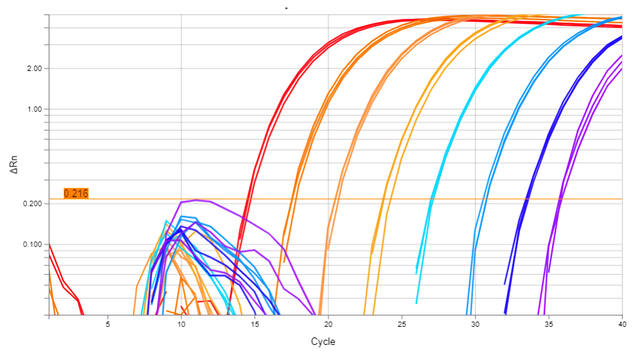
After purifying RNA from encapsulated RNA, serial dilutions were made and tested by qPCR and digital PCR (dPCR) to validate functional purity and to determine the absolute quantity. Encapsulated RNA samples were examined for stability by freeze-thaw and real-time equivalence stability tests followed by RT-qPCR.

1 en-RNA tolerates nucleases, wastewater, and saliva

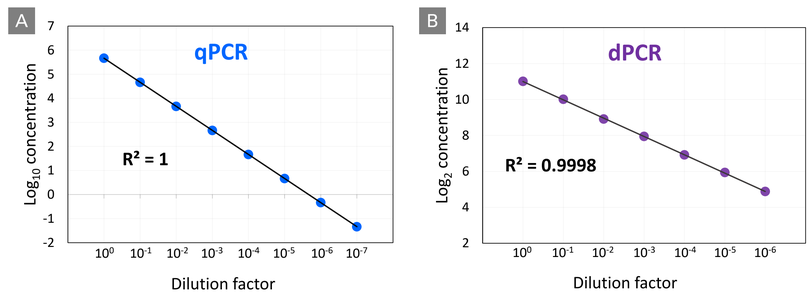


Encapsulated RNA is more resistant to degradation than naked RNA. The qPCR amplification curves demonstrate the stability of en-RNA in wastewater **(Panel A)**, saliva **(Panel B)**, and nuclease **(Panel C)** (red curves) as compared to the same en-RNA in buffer (blue curves).

2 Linearity test demonstrates purity of en-RNA

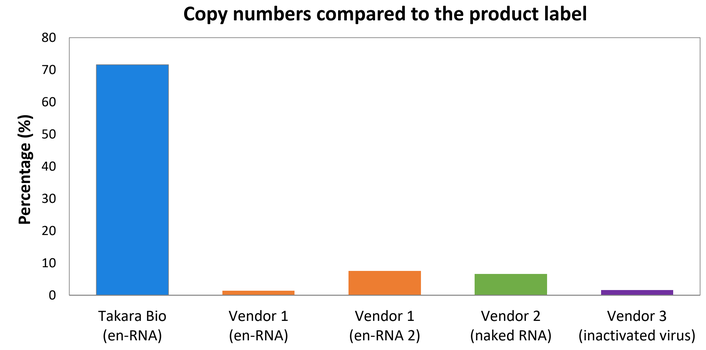


A serial dilution of en-RNA shows good linearity among different concentrations. The qPCR amplification curves produced by 10-fold dilution of SC2 en-RNA samples ranging from 10^{-3} (red) to 10^{-10} (purple) dilutions of the stock concentration.



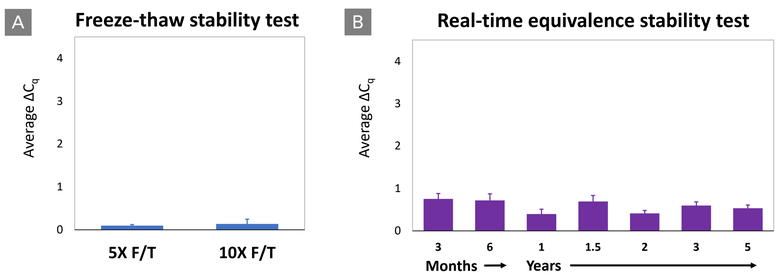
Linearity tests of en-RNA serial dilution suggest the absence of impurities and inhibiting factors. Detection by qPCR **(Panel A)** and digital PCR **(Panel B)**, show good correlation between dilution factor and concentration (copies/µl).

3 Takara Bio en-RNA delivers a more accurate quantity of RNA



A greater quantity of SC2 RNA was present in Takara Bio en-RNA control products vs. those from different vendors. By digital PCR (or qPCR, data not shown) the RNA in Takara Bio's en-RNA most closely matched the copy numbers on the product labels. All products were stored at the recommended temperature.

4 en-RNA is stable for up to 5 years



Encapsulated RNA is highly stable by two types of stability test. en-RNA subjected to 5 or 10 freeze-thaw cycles was compared to a control that experienced only one freeze-thaw cycle **(Panel A)**. By this test, the difference in C_q value (ΔCq) was <1, indicating high stability. By real-time equivalence accelerated stability test **(Panel B),** the ΔCq of timepoints up to 5 years was also <1, relative to the zero timepoint.

Conclusions

- RNA controls that are reliably stable in natural sampling environments are preferred in infectious disease and surveillance sector
- en-RNA mimics the natural virus targets being detected and quantified in molecular diagnostic and wastewater surveillance workflows
- Our en-RNA demonstrates high stability when subjected to nuclease digestion, wastewater, saliva, freeze-thaw cycles, and prolonged storage at elevated temperature
- Faster turnaround time, easy storage, flexibility, and ability to encapsulate any RNA sequence makes our product an ideal choice of RNA control

References

1. Peyret, H. et al., "Production and use of encapsidated RNA mimics as positive control reagents for SARS-CoV-2 RT-qPCR diagnostics" *J. Virol. Methods*, **300**, p. 114372 (2022).
2. Pasloske B.L. et al., "Armored RNA technology for production of ribonuclease-resistant viral RNA controls and standards" *J. Clin. Microbiol.*, **36**, 3590 (1998) .

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