2. Every precaution is taken to prevent contamination from RNases. All solutions and apparatus are treated with diethylpyrocarbonate (DEPC).
3. In vitro translation mixtures are stored at –70°C for short periods of time, and under liquid nitrogen for long periods.
4. Contaminating RNases may be removed from the linearized DNA template used for in vitro transcription by treatment with proteinase K (100 μg/mL) and 0.5% SDS for 30–60 min at 37°C before phenol:chloroform extraction.
5. The polymerase enzyme is unstable and must always be stored on ice. Concentrations of enzyme toward the upper scale of that stated in **Subheading 3.1.**, that is, 5–10 U/20-μL reaction, provide the best results.
6. Values for incorporation of radiolabeled nucleotide between 2 and 10% should be expected.
7. SDS-PAGE gel solutions may be degassed for 1–2 min before adding the ammonium persulfate and TEMED.
8. Exposure times vary, depending on radioactive label and quantity of RNA used to prime translation reactions; however, typical times are from overnight to 2–3 d.
9. A translation product obtained from a potential CP clone, which is of similar size to the CP obtained from purified virus particles, is a good indication that the clone is of full length and there are no artifically introduced frameshifts or stop codons. However, further evidence, such as immunopreciptation of the translation product with antisera against the CP, is required (*see* **Subheading 3.5.**).

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