4. Slowly stir in PEG (6%) (w/v), and continue stirring at 4°C overnight.
5. Collect the resulting precipitate by centrifugation at 10,000*g* for 15 min and resuspend in 0.5*M* borate buffer containing Triton X-100 (0.5%) (w/v).
6. Centrifuge at low speed (10,000*g* for 15 min) and transfer the supernatant to a fresh tube.
7. Sediment the virus by high-speed centrifugation (30,000*g* for 90 min) and resuspend the resulting pellet in 0.05*M* borate buffer containing 1% Triton X-100 and sonicate for 20s (21Kc/s) (*see* **Note 4**).
8. Subject the suspension to a further cycle of low- and high-speed centrifugation.
9. Purify the virus by isopycnic centrifugation in cesium chloride (0.439 g/mL, 20 h, 5°C, 110,000*g*).
10. Collect the virus band from the cesium gradient (*see* **Note 5**) and dilute at least twofold with 0.005*M* borate buffer.
11. Collect the virus by centrifugation (40,000*g* for 90 min) and resuspend the pellet in 0.5 mL of sterile distilled water (*see* **Note 6**).
12. Virus can then be used immediately, stored at 4°C for 1–2 d, or placed at –20°C for long-term storage.

3.2. RNA Extraction

All of the carlaviruses that we have worked with have yielded good quality RNA from purified particles, using a method essentially as reported by Shields and Wilson ***(11)***.

To prevent degradation when extracting RNA, gloves should be worn at all times and all tips and microcentrifuge tubes should be autoclaved to avoid nuclease contamination.

1. Add an equal volume of RNA dissociation buffer (*see* **Note 7**) to a volume of virus suspension and mix by vortexing for 5–10 s.
2. Incubate at 60°C for 3–5 min.
3. Add an equal volume of phenol, mix briefly, and incubate at 60°C for one more minute.
4. Separate phases in a microcentrifuge (12,000*g*) for 2–3 min.
5. Remove aqueous phase to a fresh tube and add an equal volume of phenol and an equal volume of chloroform and mix by vortexing for 5–10 s.
6. Separate phases in a microcentrifuge (12,000*g*) for 2–3 min.
7. Remove aqueous phase to a fresh tube and add an equal volume of chloroform and mix by vortexing for 5–10 sec.
8. Separate phases in a microcentrifuge (12,000*g*) for 2–3 min.
9. Remove aqueous phase to a fresh tube and precipitate RNA by adding 0.1 vol sodium acetate (3*M*) and 2.5 vol ethanol.
10. Store at –20°C overnight, or –70°C for 1 h, before recovering RNA by centrifugation in a microcentrifuge (12,000*g*).
11. Dry the RNA pellet under vacuum to remove all traces of ethanol before resuspending in a suitable volume of sterile distilled water.
12. Run a sample of RNA on an agarose to check yield and quality (*see* **Note 8**).