after inoculation should be used. Freezing the plant materials drastically reduces the virus yield. In the case of RRSV, no virus can be recovered after freezing of infected rice plants.

The purification protocol described here *(7)* was originally developed for maize rough dwarf virus and works well for both RBSDV and RRSV. Rice ragged stunt virus can be purified by other methods as well *(8,9)* (**Subheading 3.2.2.**).

4. At the second organic solvent treatment during the purification of viral particles, we have previously used freon. Since freon is not available anymore, carbon tetrachloride is used as a substitute (**Subheading 3.2.1., step 8**).

5. Techniques involved in extraction of dsRNAs are reviewed in detail by Dodds et al. *(15)*. Direct extraction of genomic dsRNAs described here is a modification of a method described by Dodds et al. *(10)*. The major modification is a use of CC41 *(11)*, instead of CF11 cellulose. Fine granular texture of CC41 makes it easier to handle by a batch method in a microcentrifuge tube. An additional modification is that the STE buffer does not contain mercapthoethanol, SDS, and bentonite at an extraction step. Quality of dsRNAs obtained from RDV-infectecl rice plants is pure enough to subject to RT-PCR. So far as we tested with RDV S8, S9, and S10, we have successfully amplified full-length cDNAs. We have not yet tested this protocol for RRSV and RBSDV (**Subheading 3.3.1.**).

Our standard procedure for direct extraction of the genomic dsRNAs includes additional steps. After the phenol and chloroform extraction, total nucleic acids are precipitated by ethanol and suspended in 100–400 μL of TE, and then an equal volume of 4*M* LiCl is added to precipitate high-mol-wt ssRNAs *(12)*. Supernatants containing the dsRNAs are then treated with CC41, exactly as described by Dlieu and Bar-Joseph *(11)*. The standard method has been used routinely in our laboratory for purifying genomic dsRNAs of RDV *(5)*, RBSDV, and RRSV *(13)*. The method should yield ~5–10 μg from 0.5 g of infected leaves for RDV, 0.1 μg for RBSDV, and 0.5 μg for RRSV. Using genomic dsRNA templates prepared by this method, we have successfully amplified full cDNAs of RDV S4, S5, S6, S7, S8, S9, S10, S11, and S1 2 by the basic protocol of RT-PCR described in **Subheading 3.4.1.**

A method for extraction of both viral mRNAs and genomic dsRNAs described here is based on a protocol of Chomczynski and Sacchi *(14)* (**Subheading 3.3.2.**).

References

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