Preparation of RNA from *P. infestans*

(Adapted from Loegemann, Anal. Biochem 163:16-20 (1987)

1. Harvest tissue by filtration onto Whatman 54 paper (if starting from a liquid culture; if starting from a polycarbonate membrane culture, just peel the hyphae off the membrane).
2. Grind in liquid nitrogen to a fine powder using a mortar and pestle.
3. Add powder to blender cup containing extraction buffer, and blend for 1 min. In typical extraction, use about 7.5 g "semi-wet" tissue with 25 ml buffer.

Extraction buffer: 8 M Guanidium HCl
20 mM MES pH 7.0
20 mM EDTA (from Na_2EDTA stock)
50 mM B-mercaptoethanol (add fresh)

4. Add 20 ml of phenol plus 20 ml of 24:1 chloroform:isoamyl alcohol.
5. Place on rocking platform 5 min, rt, ca. 200 rpm.
6. Spin 30 min, 10k xg, 4C
7. Save supernatant and add 5 ml phenol plus 10 ml chloroform:isoamyl alcohol. Shake 1 min, then spin 10 min 10k xg.
8. Save supernatant and add 0.012 vol glacial acetic acid (or 0.2 vol 1 M acetic acid) plus 0.7 vol precooled ethanol.
9. Store overnight at -20C or 1 hr at -80C.
10. Spin 10k xg 10 min.
11. Wash pellet twice with 3M NaOAc pH 5.2, room temperature, spinning 5 min 10x g each time (helps remove small RNAs and polysaccharides).
12. Wash with 70% ethanol (to remove salt), spinning 10 x g; air dry.
13. Resuspend in DEPC-treated water (0.5 ml). Assist by heating 5 min 90C. Remove remaining polysaccharides by placing on ice, 5 min. Spin 5 min in microfuge in cold room and save the supernatant.
14. To increase yield, repeat step 13 with the remaining pellet (up to 3 times).