Single Step RNA Isolation

You need (n= number of samples):

2 snap top/2 ml eppendorf tubes per 100 mm/60 mm plates labeled for each sample.

An additional n eppendorf tubes.

Solution D; 2 M NaOaC pH 4; Phenol (water-saturated), Chloroform-isoamyl alcohol (49:1); isopropanol.

1. Stop stimulation by placing plates on a tray of ice.
2. Add BME (2 mM from 14.4 M stock) to solution D. Add 1.8 (0.8 ml) solution D [RT] to each 100 mm (60 mm) plate. Tilt after each addition to ensure simultaneous lysis.
3. Scrape cells with a rubber policeman [sitting in DEPC water] or a sterile cell scraper into a pool at the bottom of the dish. (Anna: transfer volume to labeled tubes on ice; Azad: allow dishes to sit tilted until all cells are scraped; transfer volume to tubes at RT).
4. Add sequentially with mixing by inversion 5 times between steps (Xu: have first ingredient pre-pipetted into tubes):
    a. 200 ul (67 ul) 2 M NaOAc pH 4.
    b. 2 ml (0.8) phenol (water-saturated).
    c. 400 ul (160 ul) Chloroform-isoamyl alcohol.
5. Vortex final mix briefly and cool on ice for 15 min.
6. Cf 10Kg/20'/4ºC. (8500 RPM in SS34).
7. Transfer aqueous phase to new tube. Add 2 ml (0.8) isopropanol and cool at -20ºC for > 1 hr. [Azad: mix by pipetting up and down; don't vortex and risk spreading your material up to the side of the tubes: Anna: vortex whenever alcohol is added).
8. Ppt. @ 10Kg/20'/4ºC.
9. Redissolve pellet in 300 ul (120 ul) solution D. Transfer to eppendorf tubes. (Anna: aim solution D at pellet initially and resuspend by pipetting up and down 10 times).
10. Ppt. with 1 volume of isopropanal @ -20ºC > 1 hr.
11. Cf @ 10 min./4ºC.
12. Resuspend in 75% EtoH (1 ml), re precipitate @ -20ºC > 1 hr; Cf 10'/4ºC/14KRPM. Dry and resuspend in DepC W or T.E. 7.4. [Anna: dry pellet for 2 min in speed vac; Azad: dry samples by pipetting most of the supt off, spin again and pipette remainder. Allow to air dry for 5 min. on ice.)