Malaria Total RNA Isolation

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Harvesting the sample:

1. Resuspend culture in the flask, move to labeled 50ml tube.
2. Centrifuge the sample at 1500 rpm for 5 minutes with no brake in table top centrifuge.
3. Aspirate off the supernatant and wash the cells with 1X PBS (25ml per 1ml pellet).
4. Centrifuge the sample at 1500 rpm for 5 minutes with low brake in table top centrifuge.
5. Aspirate off the supernatant and freeze the pellet in liquid nitrogen right away (20-30 seconds).
6. Store pellet at –80°C until ready to isolate RNA.

RNA Isolation Day 1:

Before Starting: Chill TC room centrifuge to 4˚C. Label 14ml round bottom tubes "RNA Isolation, date, time, time point, initials" and put on ice [bucket 1]. Put the isopropanol on ice [bucket 2]. Make an ice + water bucket for the 50ml tubes [bucket 3]. Wear nitrile gloves due to use of phenol.

1. Thaw out the cells in 50-60°C water bath for <1 minute.
    a. Thaw all tubes at the same time (best to woFrk with no more than 6-8 at a time).
    b. Move directly into ice + water bucket as soon as thawing STARTS to occur.
2. Add 10ml TRIZOL to all tubes (still on ice + water) (Invitrogen 10296-028, stored at 4 °C) and then pipette each up and down until the cells are resuspended well (Usually about 10 times).
3. Add 2ml chloroform (Fisher BP1145-1) and vortex **1 time**. Let sit on ice for 5 minutes.
4. Centrifuge in TC room at 3000 rpm for 10 minutes @ 4˚C with NO brake.
5. Transfer the supernatant to a 14ml FALCON Polypropylene round-bottom tube using a 5ml pipette (should be about 5ml).
    a. Do this in two steps in case of contamination – do not touch bottom layer with tip.
    b. For clean-up, dump remaining "pellet" into phenol waste container, let tubes dry out in the hood for 1 or more weeks before throwing away (don't forget).
6. Add 1/10th the total volume (0.5ml) of 3M NaOAc, pH 5.5 to each tube.
7. Add an equal volume (5ml) of COLD isopropanol to each tube.
8. Vortex once to mix. Precipitate overnight at –20°C.

RNA Isolation Day 2:

Before Starting: Chill lab centrifuge to 4˚C with A-14 rotor, get bucket of ice, label 1.5ml tubes and a freezer box to go in -80 freezer. Also label 1.5ml tubes for 1:50 dilution (add 49ul DEPC water), prepare a 1% agarose gel. Wear nitrile gloves due to use of phenol.

1. Centrifuge 14ml tube at 9000 rpm (A-14 with adaptors) for 1 hour at 4°C.
    a. No lid on tubes.
    b. Load in centrifuge with labels facing out to easily find the pellet afterwards.
2. CAREFULLY pour out the supernatant into the PHENOL waste bottle (for residual trizol and chloroform). You should see a pellet at this stage.
    a. Invert the tube on a paper towel for 1-2 minutes, making sure pellet stays in place.
    b. Put the tube back on ice.

3. Wash the pellet with 10ml 70% Ethanol and vortex 1 time to mix well. Let sit on ice for 10 minutes in the fume hood.
4. Centrifuge at 9000 rpm (A-14 with adaptors) for 10 minutes at 4°C, labels facing out.
5. Carefully pour out the supernatant into a small beaker (in case pellet comes loose).
    a. Invert the tube on a paper towel for 1-2 minutes, making sure pellet stays in place.
    b. Circle pellet with marker to easily find later.
6. Dry the pellet in the speed vac until completely dry (less than 5 minutes).
    a. Taped to inside, bottom of speed vac. Speed "medium". Time "manual".
    b. Check for the smell of ethanol (should be none when dry).
    c. DO NOT put tube back on ice, keep at RT.
7. Add 100ul GOOD (DEPC) water to each tube, then go back and resuspend each pellet, one by one. Transfer each to a labeled 1.5ml tube on ice.
8. Add 100ul again to get residual RNA. Add to same 1.5ml tube on ice.
9. Take out 1ul and add to 49ul DEPC water to make diluted sample for Nanodrop.
10. IMMEDIATELY store RNA at –80°C in labeled freezer box.

RNA quantification:

1. Run each diluted RNA sample on a 1% agarose gel ASAP TO PREVENT DEGREDATION.
    a. Load 2ul dye + 10ul RNA (1:50 dilution).
    b. Load 2ul of 1kb ladder.
    c. Run @ 130V, for 15 minutes ONLY and take a picture.
        i. Should see double bands (rRNAs) between 1650 and 850bp and increasing concentration with later timepoints. Look for low molecular weight degradation.
2. Check RNA (1:50 dilution) on Nanodrop.
    a. Initialize with water, blank with water.
    b. Change sample drop down list to "RNA-40".
    c. Load sample and click "measure".
        i. Should see **peak at 260** (not 280 – protein).
        ii. Record **ng/uL** (Trophs should be ~100ug total in 100ul tube).
            1. [17.5ng/ul x 50 = 875ng/ul = .875ug/ul x 100ul = 87.5ug in tube]
        iii. Record all other measurements.
            1. **A-260** path should be between 0 and 1.
            2. **260/280** should be 1.9 - 2.0 for RNA (1.8 for DNA).
            3. **260/230** should be above 2. For readings <2, you will need to re-precipitate overnight and wash with 70% ethanol as before to reduce phenol contamination.
    d. Discard 1:50 dilution.