Modified QIAGEN RNeasy Kit Protocol

Charlie Kim

February 14, 2001

Modified 8/15/01, 10/30/01

Salmonella enterica serovar Typhimurium was giving poor RNA quality when using the standard Rneasy protocol. Some people believed it was due to poor lysis of the bacteria in the initial steps; I found that RLT alone is sufficient to eliminate all CFUs, although lysis was not specifically tested. However, lysozyme treatment does not result in any additional debris during the spin in step 3, suggesting that lysis does not occur any more efficiently in the presence of lysozyme. I also tried to look for debris by FACS and under a microscope, and could not tell any difference. Thus, the lysozyme treatment is omitted from the protocol. 8/13/01: I have re-added the lysozyme treatment. I'm still not sure if it is necessary; I will do more tests. Note that Typhimurium, as well as a number of other bacteria including Helicobacter and Campylobacter, sometimes contains intervening sequences (IVSs) in some of its 23S rRNA genes. These rRNAs are processed by RNAse III, resulting in elimination of the ~3 kb 23S band and generation of smaller bands in the 0.5 kb to 1.5 kb range.

1. Harvest 5 x 10^8 to 10^9 CFU. I use stop solution before harvesting (1/10 culture volume of 5% phenol in 200 proof ethanol).
2. Lysozyme 1 mg/ml for 3 min at room temp in 100 ul TE.
3. Add 10 ul beta-ME per 1 ml RLT just before use.
4. Add 350 ul Buffer RLT to pelleted sample, vortex 1 min.
5. Spin 2 min. This step is crucial for removing debris that seems to inhibit some later step.
6. Transfer supe to new tube, add 250 ul 200 proof ethanol.
7. Add sample to column, spin 15 sec, discard flowthrough.
8. Add 700 ul RW1, spin 15 sec, discard flowthrough. Repeat.
9. Add 10 ul RNAse-free DNAse I in 70 ul RDD. Incubate 40 – 60 min at room temp.
10. Wash with 350 ul RW1.
11. Transfer column to new tube, add 500 ul RPE, spin 15 sec.
12. Add 500 ul RPE, spin 2 min to dry, discard flowthrough.
13. Elute 50 ul Rnase-free water in elution tube.
14. Measure absorbance at 260 nm and 280 nm to determine yield and purity. I use 40 ug/ml per 1 OD unit at 260 nm for my calculations.

This protocol gives me a yield of around 30 ug of total RNA for 10^9 CFU. There is little or no genomic DNA contamination, and the A260/A280 ratio is around 1.9 - 2.1 for my good preps.