

# MICROARRAY FACILITY

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## Preparation of RNA for Affymetrix Core from Tissues

To balance the best result for RNA yield and purity, I recommend using Trizol<sup>a</sup> method followed by Qiagen RNeasy column<sup>b</sup> purification. The following protocol is based on the manufacturer's instruction with some modification indicated as underlined. For the new users, I recommend reviewing the Qiagen handbook and Trizol user instruction before proceed.

### Harvest of RNA

1. Transfer fresh tissues or frozen tissues or tissues stored in RNAlater<sup>c</sup> (picked up by forceps) in 1 ml of TRIzol Reagent per 50-100 mg of tissue.
2. Homogenize tissue using a rotor-stator homogenizer at a maximum speed with a 30-sec interval until the sample is uniformly homogeneous.
3. Incubate the homogenized samples for 5 min at RT.
4. Add 0.2 ml of chloroform per 1ml of TRIzol Reagent. Shake tubes vigorously by hand for 15 sec and incubate at RT for 3 min.
5. Centrifuge at 12,000 x g for 15 min at 4°C.
6. Transfer the aqueous phase to a fresh tube.
7. Add isopropyl alcohol into the tube at 0.5 ml per 1 ml of TRIzol used initially.
8. Incubate samples at RT for 10 min and centrifuge at 12,000 x g for 10 min at 4°C.
9. Wash the pellet with 75% ethanol, adding at least 1 ml of ethanol per 1 ml of TRIzol Reagent used initially.
10. Mix the sample by vortexing and centrifuge at 7,500 x g for 5 min at 4°C.
11. Dissolve RNA in RNase-free water completely at a concentration no less than 1 µg/µl (estimate from the pellet size if possible).
12. Measure the RNA yield by OD.

### Qiagen column clean-up

13. Pre-heat elution buffer @ 60-65 °C.
14. Aliquot no more than 100 µg RNA into a 1.5-ml tube.
15. Adjust the volume to 100 µl with RNase-free water.
16. Add 350 µl RLT Buffer (pre-add 3.5 µl of β-ME to it) to the sample. Mix thoroughly.
17. Add 250 µl ethanol (96-100%) to the lysate, and mix well by pipetting. Do not centrifuge.
18. Apply sample (700µl) to a RNeasy mini spin column sitting in a collection tube. Centrifuge for 30 sec at >10,000 rpm.
19. Reapply the sample from the flow-through onto the column. Centrifuge again.
20. Transfer the column into a new 2-ml collection tube.
21. Add 500µl Buffer RPE (make sure ethanol is added to the Buffer) and let it stand for 1 min.

22. Centrifuge and discard the flow-through.
23. Add another 500µl Buffer RPE onto the column and let it stand 1 min.
24. Centrifuge and discard the flow-through.
25. Centrifuge at maximum speed for another 3 min.
26. Transfer the column into a new 1.5-ml collection tube, and pipet with pre-heated 30 µl of elution buffer (RNase-free water) directly onto the membrane. Let it stand for 1 min.
27. Centrifuge at full speed for 1min.
28. Repeat elution again.
29. Centrifuge at full speed for 3 min.

### Prepare RNA for Core sample submission

30. Determine the RNA purity and yield by OD (260/280, 260) using a small-volume cuvette (e.g. 50 or 100 µg).
  - The OD 260/280 ratio should be close to 2.0 0 in 10mM Tris buffer, pH 7.5. If not, you may need to repeat the purification on RNeasy column again.
  - The quality of RNA should also be checked on a denatured RNA gel. The intensity ratio of 28S/18S band should be close to 2. If not, your RNA may be degraded.
  - Your RNA concentration should be of at least 1.5 µg/µl. If not, you need to continue the following step to concentrate your RNA.
  - The greatness of RNA quality is critical for the success of Microarray experiment.
31. (optional) Concentrate RNA by precipitating it with 6 µl (1/10 volume) of 3M sodium acetate, pH 5.2 and 165 µl (2.5 volume) of ice-cold absolute ethanol for at least 20 min at -80° or 2 hr at -20°C.
32. (optional) Wash the RNA pellet twice with 75% ethanol.
33. (optional) Spin down the pellet and air-dry it completely.
  - \* Do not over-dry the pellet or it may be difficult to be dissolved .
34. (optional) Re-suspend the pellet in a small volume\* of RNase-free water to make the concentration around 2 µg/µl.
  - \* For example, your total RNA yield is 100 µg. To make a concentration of 2 µg/µl, you will dissolve you RNA in 100/2= 50 µl water. Make sure the pellet is dissolved completely.
35. (optional) Measure the exact RNA concentration by OD.
  - Make appropriate dilution so your OD reading falls in the range of 0.1-0.2.
  - Take OD in a small-volume cuvette.
36. Make an adjustment of RNA concentration to **the final concentration of 1.5 mg/ml by adding appropriate RNase-free water.**
37. Aliquot your sample at 20 µg (recommended) in a 1.5-ml tube and store at -80°C. It's ready for submission.

- a BRL TRIzol Reagent Cat# 15596-026
- b Qiagen RNeasy mini kit Cat# 74104
- c Qiagen RNAlater™ Cat# 76104 (50ml) or 76106 (250ml)

\* If RNA quantity is a concern, please contact Dr. Tearina Chu @ 43873 for further assistance.