

# MICROARRAY FACILITY

Mount Sinai School of Medicine

Annenberg Building, Room 19-80

Tel: (212) 241-8194

Fax: (212) 241-1627

Protocol #: D02

Version: 2

By: Te-Hua Chu, Ph.D.

Date: 12/17/01

## Preparation of RNA for Affymetrix Core from Cultured Cells

In general and normal situation, I recommend using Qiagen RNeasy kit<sup>a</sup>. 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 before proceed.

### Harvest of RNA

1. Grow 3 plates (100mm diameter) of cells to confluence  
Many monolayer cell types contain  $1-3 \times 10^7$  cells at its confluence in a plate. Three plates usually give you a yield around 100  $\mu$ g of total RNA.  
**Note:** Cell number at confluence and RNA yield are dependent on individual cell type.
2. Prepare 6 ml of Buffer RLT by adding 60  $\mu$ l  $\beta$ -Mercaptoethanol to it.
3. Pre-heat elution buffer @ 65°C.
4. Disrupt cells by addition 2 ml of Buffer RLT onto each plate (use 4 ml if the cell number is greater than 30 million).
5. Collect cell lysate with a cell scraper and pool lysate into a RNase-free 15 ml conical (polypropylene) tube.
6. Homogenize cells in a 16 °C water bath (you can use a small beaker) using a rotor-stator homogenizer at a maximum speed with a 45 sec interval until the sample is uniformly homogeneous.  
**Note:** after this step, you may store your sample in a -80°C freezer for a few months. Before continuing processing the stored samples, incubate at 37°C for 15 minutes until the lysate is thawed completely.
7. Add 1 volume (2 ml or 4 ml according to Step 4) of 70% ethanol to the homogenized lysate, and mix thoroughly by shaking vigorously. Do not centrifuge.
8. Apply the sample, including any precipitate, to a RNeasy midi spin column placed in a 15-ml centrifuge tube. Maximum loading volume is 4 ml. Close the tube gently and centrifuge for 5 min at 3000-5000 x g. Discard the flow-through.  
**Note:** set the temperature of centrifuge @ 20°C
9. Repeat step 7 on the same spin column until all the lysate is loaded.
10. Add 4 ml Buffer RW1 to the column. Close the centrifuge tube gently and let it stand for 5 min.
11. Centrifuge for 5 min or until all the Buffer passes through the column. Discard the flow-through.
12. Add 4ml Buffer RPE to the column. Let it stand for 2 min and centrifuge. Discard the flow-through.
13. Add another 4 ml Buffer RPE to the column. Let it stand for 2 min and centrifuge. Discard the flow-through.
14. Centrifuge for additional 10 min to dry the spin-column membrane.
15. Elute RNA with 150  $\mu$ l of pre-heated elution buffer (RNase-free water). Let it stand for 1 min, and centrifuge for 3 min.
16. Repeat the elution. Let it stand for 1 min, and centrifuge for 5 min.

## Prepare RNA for Core sample submission

17. 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 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.

18. (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  $-80^{\circ}$  or 2 hr at  $-20^{\circ}\text{C}$ .

19. (optional) Wash the RNA pellet twice with 75% ethanol.

20. (optional) Spin down the pellet and air-dry it completely.

- Do not over-dry the pellet or it may be difficult to be dissolved .

21. (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.

22. (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.

23. Make an adjustment of RNA concentration to **the final concentration of 1.5 mg/ml by adding appropriate RNase-free water.**

24. Aliquot your sample at 20 µg (recommended) in a 1.5-ml tube and store at  $-80^{\circ}\text{C}$ . It's ready for submission.

a Qiagen RNeasy midi kit Cat# 75142