Sample Protocol for laser capture microdissection (from Dr. Maribel Rios):

For preparation of tissue – extract fresh brains (**no** perfusion/fixation), place in OCT-containing plastic containers and snap freeze on dry ice. Section fresh frozen tissue in cryostat (10-12 µM thick) and dry mount on superfrost plus slides (new box for RNA work only) – use gloves throughout this procedure. Store sections in -80 ^{o}C freezer.

I. In preparation for LCM, process sections as follows:

1. 100% EtOH –	1 minute
2. 95 % EtOH –	30 seconds
3. 70% EtOH –	30 seconds
4. 50% EtOH	30 seconds
5. 0.5% Cresyl violet –	1 minute
6. 50% EtOH –	30 seconds
7. 70% EtOH –	30 seconds
8. 95% EtOH –	30 seconds
9. 100% EtOH –	1 minute
10. Histoclear	1 minute

II. Dessicate completely – it is important for sections to be completely dry for LCM to work efficiently. If there is high humidity in the room (an issue during the summer), be sure to turn on the dehumidifier in the room.

III. Capture desired cells by LCM and the insert caps containing captured tissue on 0.5 ml tubes containing lysis buffer (from Picopure kit, Arcturus); turn tube upside down so that buffer covers the bottom of the cap. Process as indicated by kit's manufacturer for RNA extraction.

IV. DNase RNA

LCM RNA	_____
NEB Buffer 4	10 ul
DNAse	1.5 ul
dH_2O	to a final volume of 100 ul – incubate at 37°C for 1 hour

Following digest, bring up volume to 350 ul with dH_2O.
Add 350 ul phenol/chloroform
Place top phase in fresh tube and add 4 ug of tRNA
Precipitate RNA by adding 1/10th vol. of 3M NaAC and 2 volumes of 100% EtOH and place on dry ice for 20 minutes.
Spin down, wash pellet with 70% EtOH, spin again and resuspend in 10 ul of RNA-safe dH_2O.

V. Proceed with RNA amplification using Ambion Amp II kit.