Preparation of Tissue for Analysis:

Tissue Pulverization: For tissue pulverization, wear safety glasses. All instruments to be used in this process are pretreated for RNase activity as described in the tissue collection, pre-chilled and kept on dry ice. Remove samples stored in the 5 ml Nunc™ cryovials from the –80°C freezer and place on dry ice. Make sure a weight for the frozen sample has been recorded.

A RNase-free, auto-claved mortar and pestle are pre-chilled and kept on dry ice. Clean the mortar and pestle, and instruments between each sample. Begin by pouring a small amount of liquid nitrogen (LN_2) into the mortar. Empty the contents of the cryovial into the mortar and immediately begin pulverizing the tissue. Place one hand over the top of the mortar to prevent tissue from popping out of the mortar upon initiation of crushing. Using small tissue cubes (no larger than 2 mm^3) will minimize this occurrence. Small amounts of LN_2 are added to the sample as it evaporates. Never grind the tissue in the absence of LN_2. When the tissue has been pulverized to a fine powder, LN_2 is added to aid in the transfer of the powder to the 5 ml Nunc™ cryovial. Rinse the pestle with the LN_2 and gently swirl the mortar to collect the powder and carefully transfer to the pre-chilled cryovial. If all the LN_2 evaporates, add enough LN_2 to complete the transfer. Remember to pre-chill the cryovial on dry ice before transferring the pulverized powder. If the vial is warm, the sample will violently bubble and pop in the container. Allow all the LN_2 to evaporate and place the cap on the cryovial. Store at –80°C until ready for use.

RNA Isolation: Remove the sample from the –80°C freezer and place on dry ice. All materials used in handling the powder sample must be pre-chilled on dry ice to prevent any warming of the tissue. Weigh the powder sample, using a pre-chilled medium-sized weight boat. Chill the weigh boat by placing it on a cold metal plate on dry ice. Quickly weigh out a portion of the sample by tapping out the amount needed. Do not contaminate the sample with a spatula. Tapping the vial is the quickest and most sterile method for removing powder. Record the weight and begin RNA isolation by pouring the RLT buffer directly into the weigh boat. The powder will quickly dissolve. Transfer to a tube and begin the RNA isolation by homogenizing the tissue. Homogenization is handled exactly in the same manner as tissue. Detailed tissue homogenization and RNA isolation protocols can be found on the NIEHS Microarray Center web site (http://dir.niehs.nih.gov/microarray/method.htm).