ABI6100 RNA and DNA extraction

**RNA Tissue Storage**

Quantitative gene expression studies hinge on the preservation and quality of mRNA to obtain a measure of static-time gene expression in the study organism. The storage and collection of tissues has imposed new challenges for RNA studies. Despite these challenges quality RNA has been extracted from formalin-fixed paraffin embedded tissues that was left in storage for over 20 years (Coombs et al. 1999). However, for most studies preserving adequate and representative amounts of RNA is compromised by ribonucleases (RNases) enzymes that degrade the molecule and are ubiquitous and sturdy enough to survive freezing and autoclaving. Immersing tissue into aqueous sulfate solutions (e.g. 25 mM Sodium Citrate, 10 mM EDTA, 70 g ammonium sulfate/100 ml solution, pH 5.2 or commercially as RNALater®) precipitate degenerative RNAases and allow the tissue to be left @ room temperature for weeks, but for long-term storage samples should be maintained at -200C to -800C. If conducting experiments on living tissues and you wish to obtain a real-time estimate of gene expression, then you should use a flash freezing method by rapid immersion into liquid nitrogen or onto dry ice followed by storage into an aqueous sulfate solution (Mutter et al. 2004; Bradley et al. 2005).

**RNA extraction with the ABI6100**

Tissues from whole organisms or portions are chemically and physically digested before being transferred to the ABI6100 nucleic acid prep station. [***Applied Biosystems offers a range of protocols***](http://www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_042481.pdf)that are tailored to the type of tissue you are dealing with. This page deals with the extraction of RNA from animal tissue with the use of the Genogrinder2000 bead milling or homogenization system and the ABI6100 nucleic acid prep station. Other commercial and manual preparations of RNA isolation are available and for this I direct the reader to the ***[Ambion](http://www.ambion.com/techlib/basics/rnaisol/index.html" \o "Ambion. The Basics: RNA Isolation" \t "_blank)*** website, which covers this topic and other topics on RNA in great detail.

For a full 96-well plate prepare 60 ml of 1X nucleic acid lysis buffer (3 ml phosphate buffered saline, 30 ml 2X nucleic acid lysis solution, 27 ml ultrapure H2O). Place 5-50 mg of tissue samples into each 2 ml polypropylene deep well plate and add 600 µl of the 1X nucleic acid lysis buffer. Place cap mat onto deep well plate and strap into the Genogrinder2000 clamp assembly using a counterweighted plate for balance. Homogenize tissue samples at 1000-2000 strokes for 2-5 minutes using 2 beads per well; the actual amount of beads, strokes, and time varies from protocol to protocol. After each use of the Genogrinder2000 place two empty 96 well plates back in locked position for storage.

Centrifuge ground samples at 5700 rpm for 1 minute to bring the solution off the cap mat.  Optional: Add 50 µl NucPrep®Proteinase K solution (or 20 mg/ml proteinase K in storage buffer: 20mM Tris-HCl (pH 7.4), 1.0mM CaCl2 and 50% glycerol) to each well. Allow the plate to incubate at room temperature for 1 hour, or at 650C for 30 minutes then add 600 µl of DNA purification solution to each well. Recap the plate, vortex for 30 sections, and repeat the centrifugation step. The samples are no ready to be loaded into the purification tray.

Tranfer 100-500 µl of the homogenate to pre-filter tray that is loaded into ABI 6100 nucleic acid prep station collection position. Proceed with the purification methods outlined in the [***Applied Biosystems "Tissue RNA Isolation" protocols booklet***](http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_041236.pdf).  Separate the final elution into a working and storage stock and freeze both solutions at -20 to -800C.

For further instruction on how to use the ABI6100, contact the genetic support specialist and request to watch the instruction video.

**Assessment and quality of RNA**

A critical aspect following RNA isolation is to determine if its integrity has been compromised. The most direct method is to run the sample on an agarose gel. ***[Ambion gives a recipe, instructions, and gel image showing degraded vs. intact RNA.](http://www.ambion.com/techlib/append/supp/rna_gel.html" \o "Ambion Agarose Gel Electrophoresis of RNA" \t "_blank)*** The 3:5' assay described in Nolan et al. (2006) is designed to asses RNA integrity that may have otherwise been damaged by RNase. The method involves three amplicons stretching from the 3' to the 5' end of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA molecule that amplify with the same degree of efficiency; GAPDH is an enzyme that catalyzes an energy yielding step in carbohydrate metabolism. A qRT-PCR reaction on the GAPDH amplicons is run in parallel with the rest of the experiment. If the calculated concentrations of the three GAPDH amplicons are equal, then the RNA remained intact, otherwise, a deviant ratio shows RNA degradation.

The quantitative component of real-time PCR requires a starting concentration of RNA and cDNA, but there is no current technology that can precisely, repeatedly, and routinely quantify RNA concentrations. Technologies that are commonly employed toward this end include: gel band electrophoresis, UV spectrophotometry, Nanodrop, Ribogreen fluorescence, Agilent BioAnalyzer, and BioRad Experion. Whatever method is used needs to be reported because no two methods yield the same results (Nolan et al. 2006).

Absorbance spectroscopy is based upon the Beer-Lambert Law that defines a linear relationship between light absorbance and concentration of light-absorbing molecules. Genetic molecules and reagents exhibit characteristic absorbance maxima (e.g. 260 γmax = DNA & RNA, 270 γmax = phenol, 280 γmax = protein) and/or discerning ratios, such as A260/A280 ratios at 1.8 and 2.0 for DNA and RNA extracts respectively (Wilfinger et al (2006). While ribonucleic acid concentration is measured through a spectrophotometric absorbance ratio at 260 nm/280 nm, the absorbance reading does not give information about the integrity or amplifiability of RNA (Vandesompele et al. 2002). Absorbance readings also depend on salt concentrations, temperature, pH, and the interaction among molecules (Wilfinger et al 2006). Nonetheless, spectrophotometry is still routinely used to quantify RNA concentrations and provides a limited window of inference. Other wavelengths beyond A260/A280 can help in the study of RNA and DNA extraction purity and quality (Wilfinger et al. 2006), but  Nolan et al. (2006) give the most up-to-date and stringent protocols for measuring the integrity and quality of RNA that should be applied in each qRT-PCR experiment.

In addition to gel band electrophoresis, and UV OD260 spectrophotometer, our facility relies on the[***NanoDrop***](http://www.nanodrop.com/) ND-1000 full-spectrum UV/Vis spectrophotometer, which uses 2µl solute and provides a full spectrum (220-750nm) reading from each sample. Spectrophotometric methods give comparable results to the fluorescent ***[RiboGreen](http://www.turnerbiosystems.com/doc/appnotes/s_0043.htm" \o "Turner BioSystems RiboGreen RNA quantitation" \t "_blank)*** stain for RNA, or the ***[PicoGreen](http://probes.invitrogen.com/servlets/publications?id=137" \o "Invitrogen document" \t "_blank)*** stain for double stranded DNA quantitation when RNA concentrations are not less than 100 ng/µl (Bustin 2002). The Agilent and BioRad methods are based upon more expensive chip technologies. These assays only give estimates of total RNA or double stranded DNA, whereas relative amounts of gene expression requires targeting of mRNA specific genes of interest.

**References**

Bradley, B. J., J. Pastorini, and N. I. Mundy (2005) Successful retrieval of mRNA from hair follicles stored at room temperature: implications for studying gene expression in wild mammals. *Molecular Ecology Notes*, **5**, 961-964.

Coombs, N. J., A. C. Gough, and J. N. Primrose (1999) Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. *Nucl. Acids Res.*. **27**, e12-.

Mutter, G., D. Zahrieh, C. Liu, D. Neuberg, D. Finkelstein, H. Baker, et al. (2004) Comparison of frozen and RNALater solid tissue storage methods for use in RNA expression microarrays. *BMC Genomics*, **5**, 1-7.

**DNA extraction with the ABI6100**

Tissues from whole organisms or portions are chemically and physically digested before being transferred to the ABI6100 nucleic acid prep station. [***Applied Biosystems offers a range of protocols***](http://www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_042481.pdf)that are tailored to the type of tissue you are dealing with. This page deals with the extraction of total genomic DNA (gDNA) from animal tissue with the use of the Genogrinder2000 bead milling system and the ABI6100 nucleic acid prep station.

There is a variety of ways that organisms or tissues are preserved for research on nucleic acids, including immersion in 95% ethanol, dessication, or freezing @ -200C to -800C. Freeze samples for prolonged storage and for short term storage (< 12 hrs) the tissue may be kept at 40C.

Place 5-50 mg of tissue samples into each 2 ml polypropylene deep well plate. Prepare and add 150 µl of  NucPrep®Digestion buffer (or Qiagen buffer ATL) plus 50 µl NucPrep®Proteinase K solution (or 20 mg/ml proteinase K in storage buffer: 20mM Tris-HCl (pH 7.4), 1.0mM CaCl2 and 50% glycerol) to each well. Add 20 µl of antifoam A (Sigma); this is optional, but is useful if buffer becomes too foamy after bead milling. Place cap mat onto deep well plate and strap into the Genogrinder2000 clamp assembly using a counterweighted plate for balance. Homogenize tissue samples at 1000-2000 strokes for 2-5 minutes using 2 beads per well; the actual amount of beads, strokes, and time varies from protocol to protocol. After each use of the Genogrinder2000 place two empty 96 well plates back in locked position for storage.