Abstract # 6135

Novel Technology Used in Cancer Sample Preparation

Hamid Khoja
John Tobin
Paul Ventura
Jennifer Wu
James Laugharn

Covaris™
the sample prep advantage
Covaris, Inc. • 14 Gill Street, Unit H, Woburn, Massachusetts 01801-1721 USA
Tel: +1 781-932-3959 • Fax: +1 781-932-8705 • Email: info@covarisinc.com • Web: www.covarisinc.com

Abstract

Consistent and efficient DNA, RNA, protein, and metabolite extraction from tissues has become more in demand with the utilization of highly sensitive analysis instrumentation, requiring reproducible target sample yield and quality.

The key step of sample preparation in an ideal tissue pulverization process is to deconstruct the initial tissue sample, from a *"centimeter"* scale, to fine particles with *"nanometer"* scale fractures, so labile target molecules, such as nucleic acids, peptides and metabolites can be released from the tissue for further processing without metabolic or enzymatic degradation. Most current mechanical-based sample preparation protocols involve a combination of mechanical disruption to partially increase the surface area followed by chemical lysis. Such processes cannot effectively and efficiently complete this task as they typically break down tissue to about 100 micron particle size. Most target molecules, such as DNA, RNA, or protein, remain entrapped within the tissue mass, and cannot be extracted efficiently without diffusion and chemical lysis. The Covaris two step tissue pulverization/homogenization process is close to an ideal tissue process compared to that of other available technologies (Table 1).

We will demonstrate how to apply the Covaris proprietary AFA™ (Adaptive Focused Acoustics) technology to various sample preparation applications. We will show the material, methods, and data on how to effectively disrupt difficult cancer tissues, such as skin, bone, corneal, muscle, and release target biomolecules in a non-contact isothermal fashion to the nanometer scale. The data indicates the advantages of using the Covaris instrument over several commonly used sample preparation methods, such as pressure-based, chemical-based, shear/stress-based or non-focused, low frequency "sonicator" instruments. Using Covaris CP02 and S2 instruments, both DNA and RNA can be extracted simultaneously from the same tissue piece, with substantial higher recovery and better reproducibility. The Covaris method can be automated for higher throughput needs, and easily integrated with other reagent processes to further improve the current sample preparation methods.

Table 1: Technology/Instrument Comparison

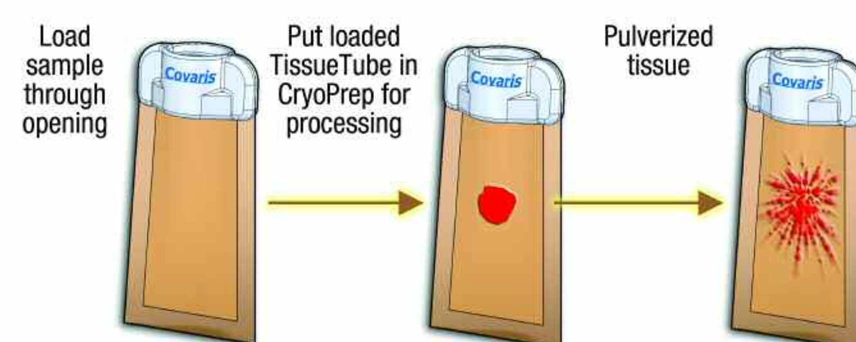
TECHNIQUE	Ideal	Dry Impact	Mortar-pestle	Bead-beater	Blender	Decompressive Shock	Sonic
SYSTEM DESCRIPTION	Ideal system	CryoPrep/S2	Mortar-pestle	FastPrep GenoGrinder	Polytron	Pressure Cycling Technology(PCT)	Sonicator
Dry Pulverization	Yes	Yes	No	No	No	No	No
Tissue Mass<5mg	Yes	Yes	No	No	No	No	No
Isothermal sample processing	Yes	Yes	Yes	No	No	No	No
Cryo-process	Yes	Yes	Yes	No	No	No	No
Samples in Tubes	Yes	Yes	No	Yes	No	Yes	No
Snap-freeze in tubes	Yes	Yes	Yes	No	No	No	No
Processing Hard Samples*	Yes	Yes	Yes	No	No	No	No
Tube Sample Storage	Yes	Yes	No	No	No	Yes	No
Labile Molecule Stability	Yes	Yes	Yes	No	No	No	No
Ease of Use	Yes	Yes	No	Yes	Yes	No	Yes
Process Speed	Yes	Yes	No	Yes	Yes	No	Yes
Batch Capable	Yes	No	No	Yes	No	No	No
No Cross-talk	Yes	Yes	No	Yes	No	Yes	No
Non-contact processing	Yes	Yes	No	No	No	Yes	No
Closed Tube processing	Yes	Yes	No	Yes	No	Yes	No
Tissue to Molecule	Yes	Yes	No	No	No	Yes	Yes

*Bone, cartilage, plants

Methodology

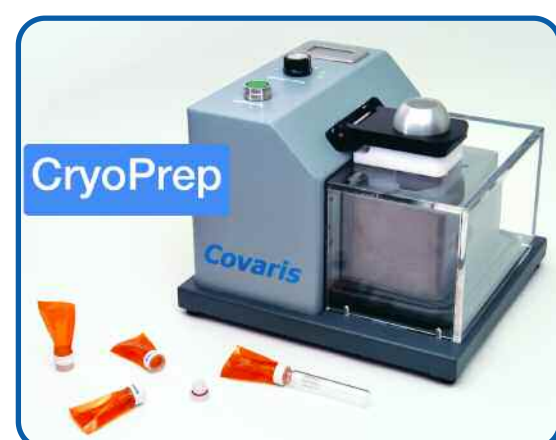
Tissue—Dry Pulverization

Covaris CryoPrep™ and Covaris TissueTubes™ (TT2) were used to both snap-freeze and freeze-fracture pulverize tumor tissue samples, following the CryoPrep operating procedures. Tumor samples (less than 0.5g) was surgically obtained, placed in tissue tube and submerged in liquid nitrogen for at least 15 seconds prior to



The sample is kept frozen throughout the entire tissue pulverization process.

placement in the CryoPrep for dry pulverization at impact level 6. The freeze and pulverization steps were repeated 3-5 times depending on tissue type. Pulverized samples in tissue bags were inverted and flick-transferred to pre-chilled Covaris 20x125 glass tube and kept cold on dry ice. Homogenization buffer was prepared by combining 3.2ml of Buffer RLT(Qiagen) with 32ul of beta-Mercaptoethanol. 3ml of RLT Buffer can be used for up to 250mg tissue. If using a cell pellet, 4-volumes RLTPlus (containing 1% mercaptoethanol) is added per 1-volume of cells.



1ml of RLT Plus beta-ME (homogenization mixture) was pipetted into the tissue bag to remove remaining tissue, then transferred into the glass tube containing the pulverized tissue. The bag was washed twice more with the remaining 2 ml RLT Plus beta-ME.

Acoustic Homogenization/Extraction

A Covaris S2 apparatus with recirculating chiller was prepared following the instrument operating procedure. The circulating chiller was set at 16°C, while the S2 water chamber is set at 18°C. After an initial 30 minute degassing of the system water, glass tubes with pulverized sample were placed one at a time in S2 for homogenization: 20% duty cycle, intensity at 10, 500 cycle/burst for 60 seconds.

The ultra-sonication acoustic protocol was repeated if any remaining unhomogenized tissue was noticed in the tube. Upon completion of homogenization, glass tubes were removed from sample holder, and 1050μl of the homogenate were aliquoted into each of three 1.5ml orange screw cap tubes from the Qiagen Allprep Miniprep kit.

DNA and RNA Purification

DNA/RNA purification were carried out using the Qiagen AllPrep Miniprep kit. Please refer to the kit manual for the complete protocol.

Each AllPrep DNA spin column has a capacity of approximately 100 μg of DNA.

Centrifuge the sample tube with homogenization mixture for 10 minutes at top speed in a microfuge. Transfer 500ul of the supernatant to an AllPrep DNA spin column placed in a 2ml collection tube (supplied). Centrifuge for 60s at >10,000 rpm. Remove and save the flow-through for RNA purification (store the flow-through at -80°C till ready for RNA purification). Continue with DNA and purification following AllPrep procedure.

Summary of Key Findings

During the latest experiments, 41 surgically obtained tissues were processed using the Covaris CryoPrep and S2, with an average DNA yield of 0.9 μg/mg of tissue and average RNA yield of 0.94 μg/mg of tissue. We believe that the DNA and RNA yield from these samples were of great improvement over previously used methods.

Our Covaris technology provides several benefits to the workflow involved in the isolation of DNA, RNA, protein, and metabolite extraction from cancer tissues:

- Our technology enables tissue samples to be snap-frozen immediately after surgical excision using the Covaris TT2 tissue tubes. Tissue pulverization is carried out quickly using the Covaris CryoPrep, and kept dry and frozen prior to the addition of homogenization buffer for stabilization. This is a critical step. Our previous findings indicate that the time between excision and freezing directly affects the yield and quality of RNA and DNA. The highest quality and yield are obtained when the tissue is frozen immediately after excision.
- The Covaris process is entirely isothermal, non-contact, and carried out in a closed vessel with no probability of cross contamination, and no cleanup. This is ideal for handling critical tissue samples, such as tumor samples in clinical settings, infectious tissue, or radioactive samples.
- The Covaris two step process of tissue pulverization and homogenization is ideal for processing the initial tissue sample to finer, dry particles, so labile target molecules, such as nucleic acids, peptides, and metabolites can be efficiently released from the tissue for further processing without metabolic or enzymatic degradation. Current mechanical-based sample preparation processes cannot complete this task effectively, as they usually only process tissue to about 100 micron particle size limit, much larger than single molecule size level (Table 1).

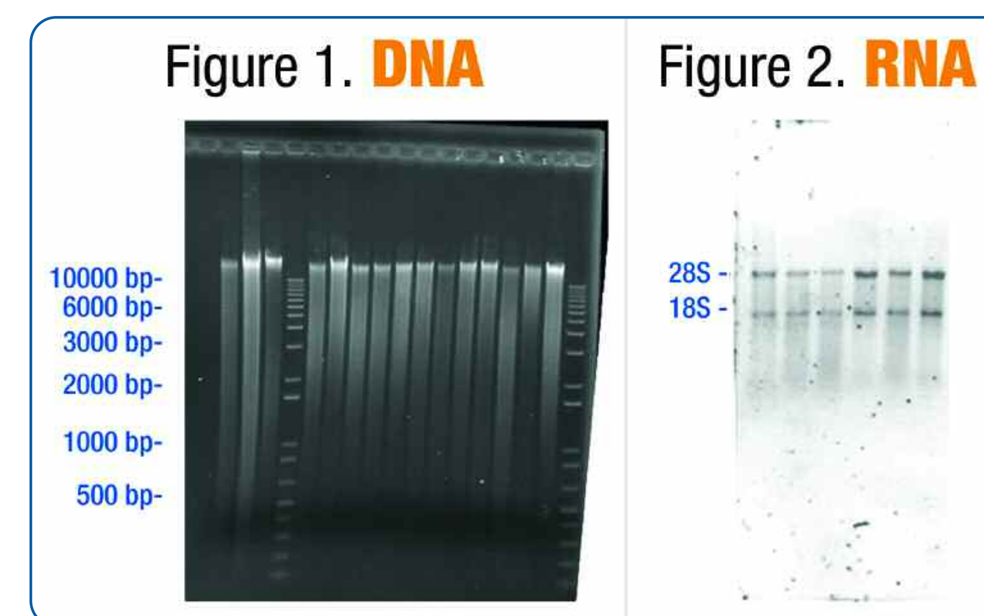
Target molecules, such as DNA, RNA, protein, and metabolites remain entrapped within the tissue mass, and cannot be extracted efficiently without diffusion and chemical lysis. Typical sample preparation processes involve a combination of mechanical disruption for the partial increase of the sample surface area, followed by a diffusion-dependent chemical stabilization with a limited effectiveness.

With the Covaris CryoPrep, we can quickly process the tissue samples to a frozen powder with nanometer scale fractures. Using the Covaris S2 AFA instrument, the frozen powder samples are then further homogenized to effectively and efficiently release high quality target molecules, such as DNA and RNA. This process also enables investigators to simultaneously extract DNA and RNA from the same sample using commercially available kits.

Results

Figure 1: Gel image of extracted DNA
Gel contains DNA extracted from colon cancer sample, a randomly chosen subset of 41 surgical tissue from different patient.

Figure 2: Gel image of extracted RNA samples
RNA extracted from the same group of colon cancer patients, an aliquot of a few samples were then analyzed for RNA on a Lonza RNA gel.



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