Non Controlled Version

**Note: Controlled Versions of this document are subjected to change without notice*

Homogenization of Tissue using Tissue Lyser LT

I. Purpose

To provide specific guidelines for using the Tissue Lyser LT to disrupt and homogenize cells or tissues. It allows the user to homogenize up to 12 samples at one time. It can be used as the homogenization method of choice when working on several samples at one time and if succeeding protocol is the All Prep-Mirvana protocol.

II. Scope

All procedures are applicable to the BCGSC Library Core and TechD Groups.

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001). Do not copy or alter this document. To obtain a copy see a QA associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Production Coordinator to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Assurance Management to audit this procedure for compliance and maintain control of this procedure.

V. References

Reference Title	Reference Number
Qiagen TissueLyser LT User Manual	IM.0213
TissueLyser LT Handbook	Qiagen 69980
AllPrep Kit Mini	Qiagen 80204
AllPrep Kit Micro	Qiagen 80284

VI. Related Documents

Document Title	Document Number
DNA/RNA Extration with AllPrep (DNA) and mirVana (total RNA with small RNA) Isolation Kits	LIBPR.0061

VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the material safety data sheet (MSDS) for additional information.

.

VIII. Materials and Equipment

Name	Supplier	Number	Model or Catalogue #	
TissueLyser LT Adapter, 12-Tube	Qiagen	69982		✓
Stainless Steel Beads, 5 mm (200)	Qiagen	69989		✓
Sample Tubes RB (2 ml) (1000 x 2 ml sample tubes, for TissueLyser LT)	Qiagen	990381		✓
Reagent DX	Qiagen	19088		✓
AllPrep Kit Mini	Qiagen	80204		✓
AllPrep Kit Micro	Qiagen	80284		✓
Fisherbrand Textured Nitrile gloves - large	Fisher	270-058-53		✓
Ice bucket – Green	Fisher	11-676-36		✓
Wet ice	In house	N/A		N/A
Nuclease Free 2.0 ml eppendorf tube	Ambion	12400		✓
Gilson P2 pipetman	Mandel	GF-44801		✓
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P20 pipetman	Mandel	GF23600		✓
Gilson P200 pipetman	Mandel	GF-23601		✓
Gilson P1000 pipetman	Mandel	GF-23602		✓
Neptune barrier tips 10 µl	CLP	Bt10XL		✓
Neptune barrier tips 20 µl	CLP	Bt20		✓
Neptune barrier tips 1000 µl	CLP	Bt1000		✓
Galaxy mini-centrifuge	VWR	37000-700		✓
VX-100 Vortex Mixer	Rose Scientific	S-0100		✓
Large Kimwipes	Fisher	06-666-117		✓

Black ink permanent marker pen	VWR	52877-310		✓
Eppendorf BenchTop Refrigerated Centrifuge 5810R	Eppendorf	5810 R		✓
Bench Coat (Bench Protection Paper)	Fisher	12-007-186		✓
Small Autoclave waste bags 10"X15"	Fisher	01-826-4		✓
Diethylpyrocarbonate (DEPC)-treated water	Invitrogen	750023		✓
DNA*AWAY*	MBS	7010		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	People Soft ID: 23878		✓
2 mL Phase lock tubes	Brinkmann	955154011		✓
TCEP, Tris(2-carboxyethyl)phosphine hydrochloride solution, 0.5M, pH7.0	Sigma Aldrich	646547-10X1ML		✓

IX. Procedure

1. Retrieval of reagents and equipment preparation

1.1. Put on a lab coat and clean pair of gloves.

1.2. Wipe down the assigned specific workstation, pipetors, and small equipment with RNAseZap RNAse Decontamination solution.

1.3. Lay down new benchcoat.

1.4. Change gloves.

1.5. Retrieve ice and all required reagents.

Note: **Tris(2-carboxyethyl)phosphine hydrochloride solution (TCEP) is highly toxic especially via inhalation. It should only be opened in a fume hood. Solutions containing TCEP should be used in the fumehood.**

2. Reagent Preparation (Including Storage Conditions)

2.1. TCEP must be added to Buffer RLT Plus before use at a final concentration of 20mM. Aliquots can be stored for up to 1 month at 4°C. These aliquots are made by the

Biospecimen Core Group. Request aliquots based on the number of samples to be processed. Consult with APC to request aliquots.

3. Homogenization

3.1. Place 2mL sample tube RB containing 1 stainless 5mm bead on dry ice for 15 min. Keep the insert of the TissueLyser LT adapter at room temperature.

3.2. Transfer up to 30mg fresh or frozen tissue to the pre-cooled tubes and incubate for another 15 min on dry ice.

3.3. Place the tubes into the insert of the TissueLyser LT Adapter, and incubate at room temperature for 2 min to avoid freezing of lysis buffer in step 3.4. (Do not incubate for longer than 2 min, otherwise the tissue will thaw resulting in potential RNA degradation)

3.4. Add the appropriate volume of buffer RLT Plus to each tube.

Note: Add to 0.5% v/v of Reagent DX to Buffer RLT Plus before starting disruption and homogenization. Use "a" option for tissue amount between 25-30mg or up to 10^7 cells and the "b" option for tissue amount of 5mg or up to 5 x 10^5 cells.

(a) 600µL Buffer RLT Plus containing TCEP to each 2mL tube and immediately place in a rack at room temperature.

(b) 350µL Buffer RLT Plus containing TCEP to each 2mL tube and immediately place in a rack at room temperature.

3.5. Place the insert with the sample tubes into the base of the TissueLyser LT Adapter, which is attached to the TissueLyser LT. Make sure that the adapter is balanced when fewer than 12 samples are to be processed.

3.6. Place the lid of the TissueLyser LT Adapter over the insert, and screw the knob until the lid is securely fastened.

3.7. Operate the TissueLyser LT for 1-2 minutes at 50Hz.

3.8. Remove the tubes from adapter and check for homogenization.

3.9. If samples are not completely homogenized, return the tubes to the insert.

3.10. Operate for another 1 minute at 50Hz.

3.11. If samples are still not completely homogenized, operate for another 1 minute at 50Hz. The duration of disruption and homogenization depends on the tissue being processed.

Note: When processing fiber-rich tissues, complete disruption and homogenization may sometimes not be possible.

3.12. Homogenization process should be kept under 5 minutes to minimize RNA degradation. Avoid overheating sample.

3.13. Remove tubes from TissueLyser.

3.14. Pulse spin tubes down briefly and transfer homogenate to a clean, labeled 1.5 mL Eppendorf tube.

3.15. Centrifuge the homogenate for 3 minutes at maximum speed (14,000-16,000x g).

3.16. Carefully remove the supernatant from each sample by pipetting, and transfer into a clean, labeled 1.5ml Eppendorf tube. Avoid aspirating any solids or debris.

3.17. Adjust the volume to either 300µL or 600µL (as indicated earlier, depending on material starting amount).

3.18. Continue with LIBPR.0061 – DNA/RNA Extration with AllPrep (DNA) and mirVana (total RNA with small RNA) Isolation Kits at homogenate transfer step to AllPrep DNA spin column.

4. Cleaning Tissue Lyser:

4.1. After finished with Tissue Lyser. Wipe down the surface of the machine with RNaseZap RNase decontamination solution. Followed by DEPC water, and then final wipe with70% EtOH.