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DNA/RNA Extraction with AllPrep (DNA) and mirVana (total RNA with small RNA) Isolation Kits

I. Purpose

To provide specific guidelines for isolating DNA and total RNA from cells and tissue.

II. Scope

All procedures are applicable to the BCGSC Library production team and Library Technology Development group.

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 Group Leader to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Assurance team to audit this procedure for compliance and maintain control of this procedure.

V. References

Reference Title	Reference Number
AllPrep Kit Mini	Qiagen 80204
mirVana miRNA Isolation Kit	Applied Biosystems (cat#AM1560)
Product Manual for Agilent 6000 RNA Nano Kit	Cat. No. 5067-1511

VI. Related Documents

Document Title	Document Number
Operation and Maintenance of Agilent 2100 for RNA Samples	LIBPR.0018
Quantifying DNA Samples using the Qubit Fluorometer	LIBPR.0030
Homogenization of Tissue using Tissue Lyser LT	LIBPR.0065

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.

Note: 1. 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. miRNA Wash Solution contains guanidinium thiocyanate which is a potentially hazardous substance and should also be used in the fume hood.

VIII. Materials and Equipment

Name	Supplier	Number: #	Model or Catalogue #	
AllPrep Kit Mini	Qiagen	80204		✓
mirVana miRNA Isolation Kit/ mirVana miRNA Isolation Kit PCI omitted	Applied Biosystems	AM1560/ AM1561		✓
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		✓
Diamond Filter tips DFL10 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171203		✓
Diamond Filter tips DFL30 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171303		✓
Diamond Filter tips DFL200 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171503		✓
Diamond Filter tips DFL1000 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171703		✓
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		✓

TCEP, Tris(2-carboxyethyl)phosphine hydrochloride solution, 0.5 M, pH 7.0	Sigma Aldrich	646547-10X1ML		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	People Soft ID: 23878		✓
Acid-Phenol:Chloroform, pH 4.5 (with IAA, 125:24:1)	Applied Biosystems	AM9720		✓
2 mL Phase lock tubes	Brinkmann	955154011		✓
BD 1ml 20G1(0.9mmx25mm)	A&B	309637		✓
BD 1ml 26G3/8 (0.45mmx10mm)	A&B	309625		✓
Reagent DX	Qiagen	19088		
Nutator Shaker	TCS Scientific		421105	✓

IX. Procedure

1. Retrieval of reagents and equipment preparation

NOTE: Ensure supervisor has requested aliquots of RLT Plus + 20mM TCEP before proceeding with the protocol.

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 RNAZap, followed by DEPC water and then finally 70% ethanol.

1.3. Lay down new benchcoat.

1.4. Change gloves.

2. Reagent Preparation (Including Storage Conditions)

2.1. Diluted TCEP is prepared by the BioSpecimen Group: Tris(2-carboxyethyl)phosphine hydrochloride solution (TCEP) must be added to Buffer RLT Plus before use. Prepare 20mM of TCEP in 1mL Buffer RLT Plus. For example, 40µL of 0.5M TCEP + 960µL Buffer RLT Plus will give you 1mL Buffer RLT Plus with TCEP.

2.2. Follow Table 1 as a guideline to determine how much RLT Plus + TCEP is required for sample processing. **Whenever the letter 'A' or 'B' is used in proceeding steps, follow the volume requirements noted in Table 1.** Consult with supervisor if unsure about the amount of RLT Plus+ TCEP is required.

Amount of Tissue/ Cells	Amount of RLT Plus + TCEP
Upto 5mg tissue	350µl (A)
Upto $5x10^5$ cells	
Upto 25-30mg tissue	600µl (B)
Upto $1x10^7$ cells	
OCT Sections	

Table 1

2.3. Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96-100%) as indicated on the bottle, to obtain a working solution.

2.4. Add 21mL 100% ethanol to miRNA Wash Solution 1 before use. Add 41mL 100% ethanol to miRNA Wash Solution 2/3. Date these solutions and write an expiry of 1 month if storing at room temperature. **For longer term storage store at 4°C, but warm to room temperature before use.**

2.5. Heat an aliquot of DEPC water at 95°C for RNA elution on a thermomixer.

2.6. Heat an aliquot of buffer EB at 70°C for DNA elution on a thermomixer.

3. Homogenization/Disruption

NOTE: All Steps using TCEP should be done in the fumehood.

3.1. **Tissue**: Only tissue samples need to be homogenized. Follow LIBPR.0065 Homogenization of Tissue using Tissue Lyser LT for homogenization of tissue samples. Proceed to Step 3.4 after completion.

3.1.1. **If the sample (Tissue) comes in TRIzol or RNAlater then it will be necessary to remove the storage medium and wash once or twice with 1mL 1x PBS buffer.**

3.1.1.1. Centrifuge at ~8000rpm for 3min

3.1.1.2. Remove PBS and depending the amount of tissue to lyse, add either **A** or **B** amount(s) of Buffer RLT Plus + TCEP. Place samples at room temperature

3.1.1.3. Proceed with tissue homogenization by following Step 3.1

3.2. **OCT Sections**: Shake the RLT Plus + TCEP (600µL) tubes with OCT material for 4 hours or overnight to dissolve the OCT media and to lyse the cells on the Nutator. Parafilm the tubes to prevent leakage. Pass the dissolved material through a syringe if any debris is present after shaking. Proceed to Step 3.5.

3.3. **Cells**: Put the tube containing the cell pellet onto wet ice once removed from the -80°C. Add the appropriate amount of RLT Plus + TCEP following the guidelines of Table 1. It is now ok to leave the sample at room temperature. Cells can just be passed through a 20G or 25G syringe (~0.9mm needle) 5-10X to disrupt the membrane. Ensure the mixture appears homogenous before proceeding to the next step. If sample has more than $1x10^7$ cells, split samples up into 2 columns or more. Proceed to Step 3.7.

3.4. Centrifuge the homogenate for 3min at maximum speed.

3.5. Carefully remove the supernatant from each sample using a pipette, and transfer it to a clean, labeled 1.5mL tube. Avoid aspirating any solids or debris.

3.6. Depending on the volume used in Step 2.2, adjust the volume of homogenate to either (A) or (B). Mix by inversion.

3.7. Transfer the homogenate to the Allprep DNA spin column placed in a 2mL collection tube. Centrifuge for 30sec at 8000 x g.

3.8. Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all the liquid has passed through the membrane.

3.9. **Do NOT discard the flow through.** Place the Allprep DNA spin column into a new 2mL collection tube and store at 4°C for later use in the DNA purification. DNA may be stored on the column up to 18 hours at 4°C. **Use the flow through for the RNA purification.**

4. Total RNA Purification using the mirVana Kit

4.1. Transfer the flow-through from each DNA column into a separate labeled 1.5mL tube. Depending on the volume chosen in Step 2.2, adjust the volume of each sample to either **A** or **B.**

4.2. Add 1/10 volume of miRNA Homogenate Additive to each flow-through, and mix well by vortexing or inverting the tube several times.

4.3. Leave the sample on ice for 10 min. Meanwhile quick spin 2mL PLG tube at room temperature for 1 minute at 14,000rpm.

4.4. In the fumehood, add equal volume to (A) or (B) of acid-phenol:chloroform to a PLG tube, ie the volume of acid-phenol:chloroform must be equal to the volume of the flow-through before the miRNA homogenate additive was added.

4.5. Add the flow through to the PLG tube and mix by inversion.

4.6. Centrifuge for 5 minutes at 10,000g at room temperature to separate the aqueous and organic phases.

4.7. Carefully remove the aqueous (upper) phase and transfer it to a fresh labeled 1.5mL tube. Note the volume removed.

4.8. Add 1.25x volumes of room temperature 100% ethanol to the aqueous phase and mix thoroughly by vortexing.

4.9. For each sample, place a filter cartridge into one of the collection tubes supplied in the mirVANA kit.

4.10. Pipet each lysate/ethanol mixture onto a filter cartridge. Up to 700µL can be applied at a time. For larger samples, apply the mixture in successive applications to the same filter.

4.11. Centrifuge for 15 seconds at 10,000 x g. **Warning:** Spinning faster may damage the filters.

4.12. Discard the flow-through, and repeat until all of the lysate/ethanol mixture is through the filter. Reuse the collection tube for the washing steps, decanting the supernatant when needed.

4.13. Apply 700µL miRNA Wash Solution 1 to filter tube and centrifuge for 10 seconds at 10,000 x g. Discard the flow-through from the collection tubes, and replace the filter tube into the same collection tube.

4.14. Apply 500µL Wash Solution 2/3 and centrifuge the filter tube for 10 seconds at 10,000 x g.

4.15. Repeat with a second 500µL aliquot of Wash Solution 2/3.

4.16. After discarding the flow-through from the last wash, replace the filter cartridge in the same collection tube and spin the assembly for 2 minutes to remove residual fluid from the filter.

4.17. Transfer the filter cartridge into a new 1.5mL collection tube (provided in the mirVANA kit).

4.18. Apply 100µL of DEPC water preheated to 95ºC to the center of the filter, and close the cap. Spin for 30 seconds at 10,000 x g (max) to recover the RNA. If multiple columns were required for a sample, combine all eluates into a single tube.

4.19. Make aliquots for Agilent QC.

4.20. Place all samples in the -80C freezer.

5. Genomic DNA Extraction

5.1. Retrieve the AllPrep DNA column from the 4ºC.

5.2. Add 500µL Buffer AW1 to the AllPrep DNA column. Close the lid gently and centrifuge for 1 minute at 14,000 x g. Discard the flow-through.

5.3. Add 500µL Buffer AW2 to the AllPrep DNA column. Close the lid gently and centrifuge for 2 minutes at 14,000 x g to wash the spin column membrane. Discard the tube containing the flow through.

5.4. Label a new 1.5mL tube to for final elution to collect the DNA with sample name and date. Transfer the DNA spin column to this collection tube.

5.5. Depending on the sample source type:

5.5.1. Add 100µL of EB (at 70ºC) to column if extracting from tissue or cells
5.5.2. Add 50µL of EB to the column if extracting from OCT sections

5.6. Incubate at room temperature for 1min.

5.7. Centrifuge for 1min at 14,000xg.

5.8. Elute DNA again by adding 50µL of EB to the spin column, regardless of sample source type. Collect the second elution in the same collection tube as the first elution.

5.9. Repeat Steps 5.6 and 5.7.

5.10. Dicard the spin column, and pool collections from the same sample if needed.

5.11. Store the DNA at 4ºC.

6. Quantification of RNA and DNA

6.1. Run aliquot of RNA sample on the Agilent for QC using the RNA nano chip. Follow the SOP for running the Agilent using the RNA nano chip LIBPR.0018 – Operation and Maintenance of Agilent 2100 for RNA Samples.

6.2. Quant the DNA following LIBPR.0030 Quantifying DNA Samples using the Qubit Fluorometer using the dsDNA High Sensitivity assay.

6.3. Proceed with LIMS if samples are to be submitted. – see Appendix A.

Appendix A

1. LIM-Scanner Protocol

1.1. Select AllPrep RNA and DNA extraction in protocol dropdown menu**.** Pipeline: SGE:GE.

1.2. Two barcodes will be generated from the protocol. The first barcode generated **will be assigned to the RNA flow through (ie mirVANA prep);** the second barcode is DNA eluted from column. Enter rack location of DNA.

1.3. Scan in DNA plate and click on "Edit Fields". Update "current volume, current volume units and sample type".

1.4. If flow-through RNA collected was taken through the Mirvana procedure, scan in the RNA barcode and run through the mirVana protocol

1.5. Scan in RNA plate and click on "Edit Fields". Update "current volume, current volume units and sample type".

1.6. Create Bioanalyzer Run for QC.

1.6.1. For Bioanalyzer Run, track protocol as "Agilent"

1.7. Scan you DNA samples and run them through the "DNA QC" LIMS protocol. Egel is not required