DNase I Treatment of RNA/Total Nucleic Acid

I. Purpose

To remove genomic DNA from RNA or Total Nucleic Acid (TNA) preparations

II. Scope

All procedures are applicable to the BCGSC Library Core and the Library 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 Quality Systems 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 Systems Management to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
N/A	N/A

VI. Related Documents

Document Title	Document Number
Operation and Maintenance of the Agilent 2100 Bioanalyzer for DNA samples	LIBPR.0017
Automated DNA Quantification using the dsDNA Quant-iT High Sensitivity Assay Kit and VICTOR3V/VICTOR X3	LIBPR.0108
Operation and Maintenance of the Agilent 2100 Bioanalyzer for RNA Samples	LIBPR.0018
Operation and Maintenance of the LabChipGX for RNA samples using the HT RNA Assay	LIBPR.0052
Total RNA Normalization on the Hamilton Nimbus	LIBPR.0121
Operation and Maintenance of the LabChipGX Touch HT for RNA samples using the High Throughput RNA Assay	LIBPR.0184

Document Title	Document Number
Quantifying RNA Samples Using the Qubit 4 Fluorometer	LIBPR.0186
Quantifying DNA samples using the Qubit 4 Fluorometer	LIBPR.0153

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 safety data sheets (SDS) for additional information.

VIII. Materials and Equipment

Name	Supplier	Number
Fisherbrand Textured Nitrile gloves - large	Fisher	270-058-53
RNAse Zap	Ambion	9780
Ice bucket – Green	Fisher	11-676-36
Wet ice	In house	N/A
RNAse free 1.5mL 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
Mandel P200 DF200 tips	Mandel	GF-F171503
Mandel P1000 DF1000 tips	Mandel	GF-F171703
VX-100 Vortex Mixer	Rose Scientific	S-0100
200µL Rainin tips	Rainin	RT-L200F
20µL Rainin tips	Rainin	RT-L10F
200µL Pipet-Lite	Rainin	L12-200
20µL Pipet-Lite	Rainin	L12-20
1250µL pipette tip, 96tips/rack, 480 tips/cs filter sterile	Mandel Scientific	TM-4445
Pipette-VIAFLO 8 Channel	Mandel Scientific	TM-4124
Large Kimwipes	Fisher	06-666-117
Black ink permanent marker pen	VWR	52877-310
Bench Coat (Bench Protection Paper)	Fisher	12-007-186
Small Autoclave waste bags 10”X15”	Fisher	01-826-4
DNAse1 Amplification Grade 100U	Invitrogen	18068-015
DEPC water	Ambion	9922
Mini-centrifuge	Eppendorf	5417R

Name	Supplier	Number
Thermo Scientific 0.2mL Ultra Rigid Skirted 96-well	Thermoscientific	AB1000-150s
Deep-well, 96-well, 1.2mL, U bottom, low pro, 50/cs	Fisher Scientific	AB1127
RNA MagClean DX	ALINE Biosciences	C-1005
Sterile Filtered Conductive 50µL Tips in Frames	Hamilton	235979
Sterile Filtered Conductive 300µL Tips in Frames	Hamilton	235938
Tape Pads	Qiagen	19570
Foil Tape	VWR	60941-126
PCRmax Alpha Cycler 4	Froggabio/Cole Parmer	AC496
2100 Electrophoresis Bioanalyzer Instrument	Agilent	G2939AA
LabChip GXII	Perkin Elmer/Caliper	124582
LabChip GX Touch	Perkin Elmer/Caliper	CLS137031/E
NIMBUS Liquid Handling Workstation	Hamilton	

IX. GENERAL GUIDELINES

1. General guidelines

1.1. The beads (**RNA MagClean DX**) to be used in this protocol are different from those used for other protocols as they are certified to be RNase-free.

1.2. Ensure proper personal protective equipment is used when handling sample plates, reagents and equipment. Treat everything with, and adhere to, strict RNA handling techniques.

1.3. Wipe down the assigned workstation, pipetman, tip boxes and small equipment with RNAse Zap (Ambion) followed by DEPC-treated water. Ensure you have a clean working surface before you start.

1.4. Double check the QA release and/or expiry date of each reagent and enzyme.

1.5. Reactions in plates should never be vortexed and plate covers are never to be re-used.

1.6. Retrieve and thaw all reagents at room temperature. Once thawed, pulse-vortex, quick spin, and keep reagents on ice. Enzymes should be left in the freezer until ready to use.

1.7. Ensure the waste bag for the Nimbus is empty.

1.8. Brews are prepared and dispensed manually. Note that what is dispensed into the final brew plate is the actual volume of the specified volume for each step below without any dead volume.

1.9. Beads are manually dispensed into wells in a 1.2mL plate (AB1127). 20µL dead volume should be factored in, which means you need to dispense 110µL in the final bead source plate that will be placed on the Nimbus. Ensure that you include 25mL dead volume for 70% ethanol and DEPC H_2O on top of what is required for the actual washes and elution, respectively.

1.10. The Nimbus adds sample or sample in a given reaction to the brew and bead cleanups are performed on Nimbus. Follow the prompts and lay out from the Nimbus programs to execute a particular step.

1.11. The Nimbus mixes at 80% of total volume 10 times.

1.12. Note that where it is specified that you proceed immediately to the next step, plates can be briefly placed on ice (not more than 30 minutes) in the case of emergency. Make an active attempt to proceed as immediate as possible.

X. PROCEDURE

Note: All version numbers for Nimbus protocols have been removed on this document. If you are unsure which version to use, consult your supervisor.

1. Upstream Preparation

1.1. In the case that LIMS is down, brew calculators can be located on the worksheet listed below. Enter the number of samples to be processed and print the DNase Worksheet located in:

R:\Library Core\Work Sheets and Calculators\DNase Treatment

1.2. Retrieve the plate containing Total RNA or TNA. If stored in -80°C, thaw it on ice followed by a quick spin at 4°C, 700g for 1 minute. Place it on ice.

2. Input QC:

2.1. For <u>total nucleic acid</u>, it is recommended that contaminating gDNA is first quantified using Qubit (LIBPR.0153) or Quant-IT (LIBPR.0108). The RNA/DNA mixture entering this DNase treatment should contain <400ng gDNA. Normalization to 200-400ng gDNA should be performed using Nimbus (LIBPR.0121) or manually if deemed appropriate.

2.2. For RNA extracted using protocols such as Qiagen's where RNA is separated from gDNA, gDNA quantification is not required.

3. DNase I Treatment: Remove contaminating gDNA in samples

Depending on the extent of gDNA content, the downstream application and the nature of the sample, 1, 2 or 5 Units of DNase is used (Consult your supervisor if unsure). Samples should be in 35µL.

Solution	µL (per 1rxn)
RNA	35
DEPC H20	5, 8,or 9
10X DNase Buffer	5
DNase I Enzyme	1, 2 or 5
Total Reaction volume	**50**

} 1st DNase Mix (15µL)

Pipeline	Sample input	Amount of DNase I
SSTRA version 3,4	Intact RNA	1U
SSTRA version 3,4	Total nucleic acid	5U
RBD version 2,3	Total nucleic acid	2U
CLINxGen pipeline	Intact RNA (from CGL)	5U
CLINxGen pipeline	FFPE RNA (from CGL)	No treatment
CLINxGen pipeline	Fresh Total nucleic acid	5U
CLINxGen pipeline	FFPE Total nucleic acid	5U

3.1. Make sure to mix each reagent well and quick spin before adding to the brew. Add enzyme last.

3.2. Prepare the reaction brew in a non-stick tube according to amount of DNase I listed in the above table and check off reagents as they are added on the worksheet. Mix the brew by repeated pulse-vortexing followed by a quick spin. Use the appropriate calculator from the following:

LIMS Calculator: DNase_1U
LIMS Calculator: DNase_2U
LIMS Calculator: DNase_5U

3.3. Log into Nimbus Program as follows to add the RNA to 15µL of the DNase mix:

Open file: **Production** > *toggle to workflow* > **DNase I Treatment Scheduler.wfl > DNase I Addition**

3.4. After Nimbus program completion, seal the plates and quick spin at 4°C for 1 minute. Inspect the reaction plates for any variations in volume.

3.5. Incubate for 15 minutes at room temperature.

Note: *This is **NOT** a safe stopping point; proceed to the next step immediately*.

4. Post–DNase I treatment Bead clean up (use RNA MagClean DX beads)

4.1. The input volume for this step is 50µL per well.

4.2. The Nimbus will perform the cleanup of the 1st DNase reaction using beads as follows:

Reaction	*Bead Vol manually dispensed (µL)	Bead Vol added by Nimbus (µL)	Bead Binding Time (mins)	Magnet Clearing Time (mins)	2X 70% EtOH* Wash Vol (µL)	Magnet Airdry Time (mins)	DEPC Elution Vol (µL)	Elution time (mins)	Magnet Elution time (mins)	Transfer Vol (µL)
1st DNase Reaction	**110**	**90**	**15**	**7**	**150**	**5**	**10**	**3**	**2**	**10**

*Must be at Room Temp for a minimum of 30 minutes before usage; failure to do so would result in a decrease in yield

4.3. Log into Nimbus Program as follows:

Open file: **Production** > *toggle to workflow* > **DNase treatment > DNase I Treatment Scheduler.wfl > Bead Clean DNase I**

*This is a safe stopping point. If needed, the plate containing the bead cleaned RNA can be stored at **-80°C.***

5. QC of DNase treated RNA : Follow the relevant upstream/downstream SOP(s) or consult your supervisor

Note: for clinical samples there in no QC at this step.

Appendix A: LIMS SOP

1. Start of Plate Library Construction

2. **For clinical samples only:** CxG DNase I Treatment - **CLC1.0CSPC1.1 pipeline**

3. **For all other samples:** DNase Treatment: your supervisor will provide you with the pipeline.

4. Bioanalyzer Run / Caliper Run – if working with intact RNA: QC samples on Agilent after 1st DNase treatment. Create Bioanalyzer Run – QC Category: Total RNA QC **(not for clinical samples)**

   RNA Qubit Assay: if working with FFPE RNA or FFPE total nucleic acid that has been DNase treated in set less than 24 samples. LIMS: **RNA Qubit QC (not for clinical samples)**

   OR

   Caliper Run – if working with FFPE RNA or FFPE total nucleic acid that has been DNase treated in set greater than 24 samples. QC Category: Total RNA QC **(not for clinical samples)**